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INDIAN AGRICULTURAL
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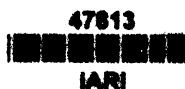
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TRANSACTIONS

Studies on Vitamin B₂ Complex. V

Further Experiments on the Effect of Carbohydrate on
Vitamin B₂ Deficiencies. Flavin Synthesis in Rats.*

By Ume TANGE.

(The Institute of Physical and Chemical Research.)

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In the previous paper⁽¹⁾ data were submitted which showed that the type of carbohydrate employed in the basal rations was an important factor in the study of the vitamin B₂ complex. The remarkable difference in the development of the vitamin B₂ deficient symptoms in the rats fed with the diets containing sucrose, corn-starch and lactose was noticed. Especially, in the case of the lactose ration, none of the rats did develop dermatitis, although showing cataract, and they attained somewhat subnormal growth even in the entire absence of vitamin B₂ complex. These results suggested that the presence of lactose might have favoured the synthesis of vitamin B₂ complex by the bacterial flora in the rats' intestine. Moreover, the past experiments⁽²⁾ with diets containing dextrin and sucrose, deficient in vitamin B₂ but otherwise complete, showed very different effects on the onset of dermatitis; namely, that no dermatitis occurred with the dextrin ration but with a similar sucrose diet dermatitis was quite severe.

These and other observations led the author to attempt further investigations to determine whether rats can synthesize vitamin B₂ factors when the experimental diets are deficient in these factors.

EXPERIMENTAL.

The series of diets employed in the present studies was similar in composition to those previously reported,⁽¹⁾ as shown in Table I.

The methods employed in this experiment were mostly similar to those described in the previous paper,⁽¹⁾ care being taken to distribute litters and sex uniformly throughout the several groups. The young rats weighing between 45

Table I.

Composition of various rations used:

| Component \ Rations (per cent.) | Diet C | Diet S | Diet L | Diet G | Diet D |
|--|--------|--------|--------|--------|--------|
| Purified fish protein | 18 | 18 | 18 | 18 | 18 |
| McCullum's salt mixture | 4 | 4 | 4 | 4 | 4 |
| Agar-agar | 1 | 1 | 1 | 1 | 1 |
| Crisco | 9 | 9 | 9 | 9 | 9 |
| Corn-starch (commercial) | 68 | — | — | — | — |
| Sucrose (<i>Pharmacoepia Japonica</i>) | — | 68 | — | — | — |
| Lactose (" ") | — | — | 68 | — | — |
| Glucose (" ") | — | — | — | 68 | — |
| Dextrinized corn-starch† | — | — | — | — | 68 |

† Made from commercial corn-starch by moistening the starch with a 0.1 per cent. solution of citric acid, autoclaving for 5 hours at 120°C, drying and pulverizing.

to 55 g were placed in the cages provided with raised bottoms of coarse wire-screens to prevent accessibility to feces. When the weight of the animals remained stationary or declined, one drop of cod liver oil and 20γ of vitamin B₁ hydrochloride were supplied daily.

In the case of the diets with the lactose and dextrinized cornstarch, a number of animals was placed on these diets in advance of the remaining groups in order that their feces might be available as supplements to the other groups of vitamin B₂ complex deficient diets. The growth curves of the former groups of rats were not shown in Charts, but they were similar to those of the lactose- and dextrin-diet groups, given in Charts 1 and 2.

The feces excreted by the animals receiving the lactose and dextrin rations were collected daily and stored under ether until adequate amounts were obtained. Then the feces were extracted with ether several times to remove fatty materials and pulverized. These pulverized feces were provided at level of 0.5 g daily as supplements to the vitamin B₂ complex deficient diets containing other carbohydrates than lactose. The results are shown in Charts 3 to 10.

One experiment was carried out as a continuation of the effect of lactose on the cataract-producing action. Some groups of rats were fed on a diet similar to Diet L given in Table I, but with 55% lactose and 35% fish protein or egg albumin instead of 68% lactose and 18% fish protein, supplemented likewise with vitamins B₁, A, and D. Very few of these rats showed cataract which was less complete and was greatly delayed in development. However, the growth rate of the animals was not high, but rather low, compared with that on 68% lactose and 18% protein. The addition of filtrate factor brought about obvious improvement on

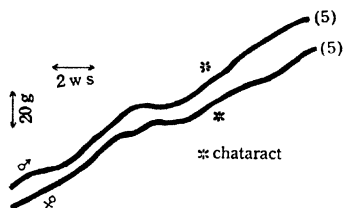


Chart 1. Average growth curves of rats on Diet L, without feces. The figures in brackets denote the number of animals considered.

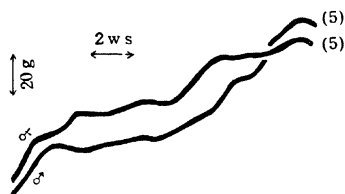


Chart 2. Average growth curves of rats on Diet D, without feces. The figures in brackets denote the number of animals considered.

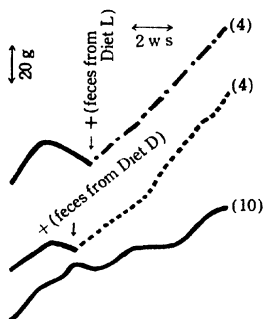


Chart 3. Average growth curves of rats on Diet D, with or without feces. The figures in brackets denote the number of animals considered.

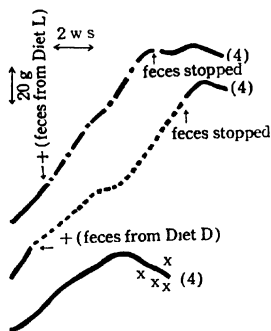


Chart 4. Average growth curves of rats on Diet C, with or without feces. The figures in brackets denote the number of animals considered, x died.

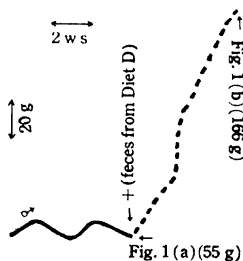


Chart 5. Growth curve of rats on Diet C, supplemented with feces from Diet D rats. (Refer to Figs. 1, (a) and (b).)

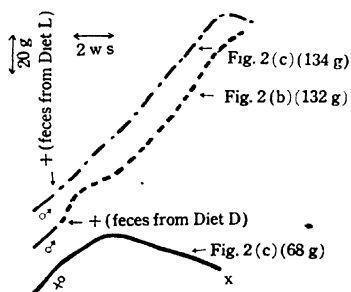


Chart 6. Growth curves of rats on Diet C, with or without feces. x died. (Refer to Figs. 2, (a), (b) and (c).)



Fig. 1. (a) Showing the subnormal condition of the rat on Diet C, not supplemented with feces (body wt. 55 g).



(b) Showing the normal health and growth after about 5 weeks of administration of the feces from Diet D rats on the same rat (a) (body wt. 166 g).



Fig. 2. (a) Showing the subnormal condition of the rat on Diet C, not supplemented with feces.



(b) Showing the influence of feces from Diet D rats on the rat fed with Diet C, to promote growth and improve health.



(c) Showing the influence of feces from Diet D rats on the rat fed with Diet C, to induce normal health and growth.

These rats are litter mates. They were photographed on the fifty-fourth day of the experiment, at which time they weighed 68 g (a), 132 g (b), and 134 g (c), respectively.

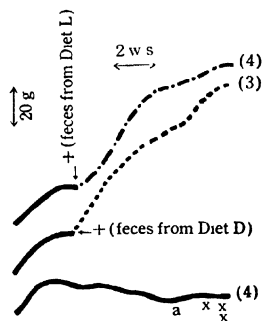


Chart 7. Average growth curves of rats on Diet G, with or without feces, a acrodynia, x died. The figures in brackets denote the number of rats considered.

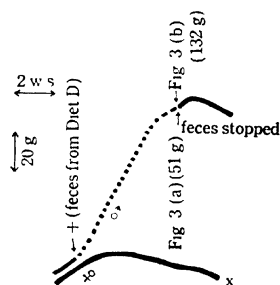


Chart 8. Growth curves of rats on Diet G, with or without feces, (Refer to Figs. 3, (a) and (b).)



Fig. 3. (a) Showing the unhealthy appearance of the rat on Diet G, not supplemented with feces.



(b) Showing the favourable growth effect of the feces from Diet D rats on the rat fed with Diet G.

These rats are litter mates. They were photographed on the forty-second day, at which time they weighed 51 g (a) and 132 g (b), respectively.

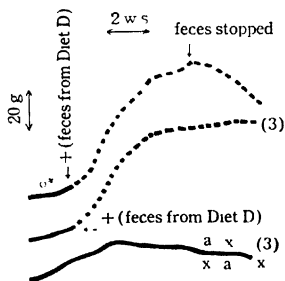


Chart 9. Average growth curves of rats on Diet S, with or without feces, a acrodynia, x died. The figures in brackets denote the number of rats considered.

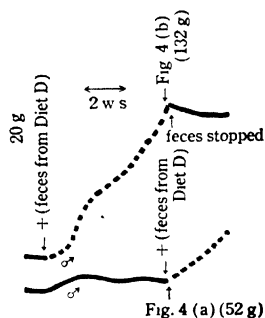


Chart 10. Growth curves of rats on Diet S, with or without feces, (Refer to Figs. 4, (a) and (b).)

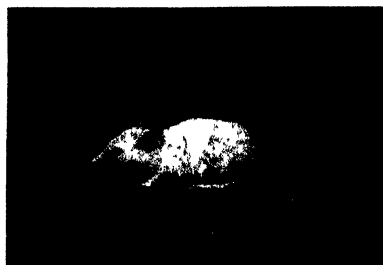


Fig. 4. (a) Showing the unhealthy appearance of the rat on Diet S, not supplemented with feces,



(b) Showing the favourable growth effect of the feces from Diet D rats on the rat fed with Diet S.

These rats are litter mates. They were photographed on the forty-ninth day, at which time they weighed 52 g (a) and 132 g (b), respectively.

the growth. In this case, the inhibitory action on cataract was attributed to the effect of the amount of protein rather than that of lactose, since 55% lactose and 18% protein in a similar diet had nearly the same degree of cataract production (unpublished) as in 68% lactose and 18% protein.

Fully developed cataract occurred in nearly 100% with the ration containing 68% lactose and 18% fish protein and the average time required for its production was 10 weeks, while with the ration containing 55% lactose and 35% fish protein or egg albumin, the cataractous change in the lens appeared in only insignificant degree in the experimental period of about 18 weeks, except in two out of sixteen rats which showed marked cataract. These relations are shown in Chart 11.

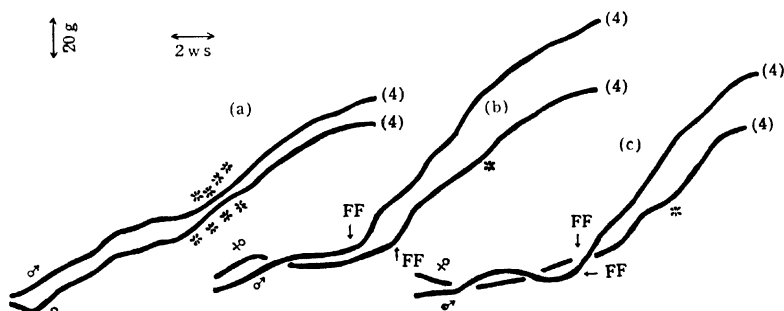


Chart 11. Average growth curves of rats on diets containing 18% fish protein (curve a), 35% egg albumin (curve b), and 35% fish protein (curve c), respectively.

FF filtrate factor, * mature cataract. The figures in brackets denote the number of animals considered.

RESULTS AND DISCUSSION.

From the above results, it appeared evident that the groups of rats which received feces, excreted by the animals fed with lactose- and dextrin-diet showed a much more favourable growth response than comparable groups of animals which received the respective diets unsupplemented. This beneficial effect of lactose and dextrinized corn-starch was considered to be due to the action of the intestinal flora of the rats. With the examination of the feces this condition was found to be true. We observed a remarkable contrast in the nature of the feces excreted by the animals receiving the lactose and dextrin diets, compared to the feces voided by the animals receiving sucrose-, glucose-, or corn-starch-diet. The feces from the animals fed on the former two diets (lactose and dextrin) were usually large, moistened, bulky pellets, while those from the animals fed on the latter three diets (sucrose, glucose, and corn-starch) were hard, small, black pellets of commonly irregular form. It seemed, therefore, to be possible that the different nature of these two sets of groups mentioned above, was attributable to the results of the action of certain microorganisms that inhabited the digestive tract of the animals. On autopsy examination of such animals, the cecum of the lactose- and dextrin-fed was unvariably found to be distended and filled with residual dietary materials, in contrast to the contracted and empty cecum of the animals which received the sucrose, glucose, or corn-starch as the source of carbohydrate.

As the results of the above finding, an attempt to isolate flavin from the feces was made with the hope of ascertaining the components of vitamin B₂ implicated. The determination of flavin in the feces was made by the methods of Kuhn.⁽³⁾ The ether extracted residue (about 60 g as dry powder) of feces mentioned above, was extracted three times with 80% methanol. The combined methanol solution was concentrated under reduced pressure, and this concentrated solution was extracted first with ether, and then with chloroform to remove fatty materials and pigments. After the aqueous solution was separated from ether and chloroform, it was acidified with HCl to pH 3.0, and then adsorbed twice on acid clay. The united adsorbates were eluted by shaking with a mixture of pyridine-methanol-water (1:1:3). After similar treatment was repeated twice more, the eluates were combined and evaporated in vacuo until pyridine was completely removed. These procedures were all carried out protected from light. The evaporated residue was diluted with H₂O and made to 0.5 N alkaline solution with NaOH. This alkaline solution was irradiated by passing air current on 500 W electric lamp at a distance of 20 cm below 20°C for 2 hours. The resulted lumiflavin was acidified with HCl, and extracted several times with CHCl₃. The combined chloroform extracts were dehydrated with anhydrous sodiumsulphate and concentrated to a definite volume under diminished pressure, then lumiflavin was estimated by Zeiss' Pulfrich Photometer. The amounts of flavin calculated from lumiflavin were about 300 micrograms. Their absorption spectra are given below.

Such findings as above led the author to determine whether the respective

rations used in these experiments carried appreciable amounts of flavin and whether the presence of untreated corn-starch in the rats' intestine favoured also the bacterial flora to synthesize flavin. It was found, however, that none of them contained flavin. This indicated clearly that the diets containing lactose and dextrinized corn-starch affected the intestinal activities very differently from those containing sucrose, glucose, and corn-starch as source of carbohydrate. It was highly interesting to find that the dextrinized corn-starch had a favourable influence on bacterial flora in the rats' intestine, and that the untreated corn starch failed to show any such properties.

Thus data showed quite conclusively that the beneficial effects of lactose and dextrinized corn-starch on vitamin B₂ deficiencies were to be attributed to the nature of these two carbohydrates to favour the production of these factors by microorganisms in rats' intestine. Flavin was isolated from the feces from the animals fed with the two diets. It was not correct to judge, however, that flavin was the only factor contained in the feces, since sufficient amounts of vitamin B₂ factors were supplied by the feces to produce satisfactory growth comparable to that obtained by providing flavin, B₆ and "filtrate" factor to similar vitamin B₂ deficient diets as shown in the previous experiments.⁽¹⁾ When the feeding of feces was stopped, the animals declined in weight, with poor appearance of the fur and skin. Guerrant⁽²⁾ et al. showed that live yeast cells existed in the cecum of dextrin-fed rats in abundance, and those microorganisms were the specific agents to produce the B vitamins. Moreover, Bechdel and his co-workers⁽³⁾ isolated bacteria from the dried matter of cows' rumen and designated them *Flavobacterium Vitarumen*, and the microorganisms had very high ability to synthesize the vitamin B complex.

Absorption spectra.— The absorption spectra of the lumiflavin obtained from the feces, voided by the animals fed on lactose and dextrinized corn-starch diets, were very similar to those of lactoflavin estimated by Kuhn,⁽⁴⁾ even though the maximum points were not distinct like the pure lactoflavin, which might be due to contamination by some impurities. They are given below for the comparison:

| | | |
|--|--|---|
| $\lambda=4450 \text{ \AA}$ | $\lambda=4450 \text{ \AA}$ | $\lambda=4450 \text{ \AA}$ |
| $\lambda=3650 \text{ \AA}$ | $\lambda=3800 \text{ \AA}$ | $\lambda=3770 \text{ \AA}$ |
| $\lambda=2850 \text{ \AA}$ | $\lambda=2900 \text{ \AA}$ | $\lambda=2700 \text{ \AA}$ |
| $\lambda=2200 \text{ \AA}$ | $\lambda=2600 \text{ \AA}$ | $\lambda=2500 \text{ \AA}$ |
| H ₂ O solution of lactoflavin. By Kuhn. | CHCl ₃ solution of lumiflavin from feces of lactose diet (Fig. A). By the author. | CHCl ₃ solution of lumiflavin from feces of dextrinized corn- starch diet (Fig. B). By the author. |

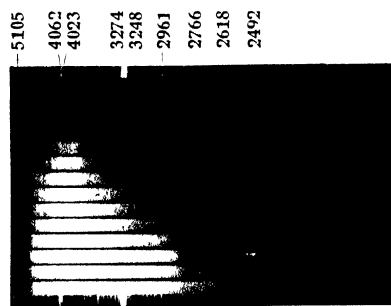


Fig. A. $1/30000$ M. CHCl_3 solution of lumiflavin isolated from the feces on Diet I, rats.

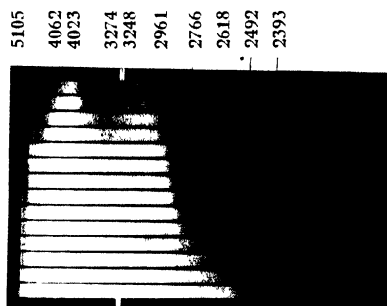


Fig. B. $1/30000$ M. CHCl_3 solution of lumiflavin isolated from the feces on Diet D rats.

SUMMARY.

1. Data are presented which show that in the vitamin B_2 deficiency the groups of rats receiving feces, voided by the animals fed on lactose and dextrinized corn-starch diets, showed a much more favourable growth response than comparable groups of animals receiving sucrose, glucose, and corn-starch.

2. The peculiar properties of the lactose and dextrinized starch are attributed to the formation of vitamin B_2 factors by microorganisms in the intestine of the rats.

3. Flavin is isolated from the feces of such rats.

4. Evidence indicates that the increased level (35%) of either fish protein or egg albumin more greatly inhibits the cataractous change in the lens than 18% level of protein.

I wish to thank Prof. U. Suzuki and Prof. B. Suzuki for their advice and encouragement during the progress of this work, and to Dr. M. Sumi for his helpful suggestions. I am also very grateful to Dr. S. Kato for the spectroscopic assay, to Miss T. Akaho for the lumiflavin determinations, and to Dr. Y. Akutagawa, of Medical Department of Jikei University, for the ophthalmoscopic study on the lens change of the animals. I am indebted to Misses M. Takahashi and H. Sasaki for their willing help in feeding the animals and preparing the materials.

* This paper was presented at the Scientific Meeting of I. P. C. R., June 16, 1939.

(1) U. Tange: Sc. Pap. I. P. C. R., **35**, 64 (1939).

(2) U. Tange: Rikwagaku-kenkyu-jo Iho, **16**, 1058 (1937).

(3) R. Kuhn, T. Wagner-Jauregg and H. Koltschmitt: Ber., **67**, 1452 (1934).

(4) N. B. Guarrant and R. A. Dutcher: J. Biol. Chem., **110**, 233 (1935).

(5) S. I. Bechdel, H. E. Honeywell, R. A. Dutcher and M. H. Knutsen: J. Biol. Chem., **80**, 231 (1928).

(6) R. Kuhn, P. György and T. Wagner-Jauregg: Ber. Chem. Ges., **66**, 1035 (1933).

ABSTRACTS

from

TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

**On a New Polypeptide Isolated from *Eisenia Bicyclis*.
(Part II)**

A Study of the Chemical Structure of Eisenin. (1)

(pp. 1~6)

By Toshihiko OOHIRA.

(Agricultural Chemical Laboratory, Tokyo Imperial University;

Received Dec. 5, 1939.)

It has been described in the previous paper that "Eisenin", a tripeptide isolated from *Eisenia Bicyclis*, has the composition $C_{13}H_{20}O_6N_4$, and yields glutamic acid (2 mols), alanine (1 mol) and ammonia (1 mol) as the ultimate hydrolysis products. It has also been confirmed that eisenin contains each one of free carboxyl- and acidamide-group.

In the present paper, some noteworthy data for determining the chemical structure of eisenin are reported.

When eisenin was heated with 3% aqueous barium hydroxide solution on a boiling water-bath, its partial hydrolysis took place, evolving almost quantitatively one equivalent of ammonia and leaving a syrupy substance which gave ninhydrin reaction contrary to the original substance and still intensive biuret reaction.

By estimating acidity and amino nitrogen content of the syrupy substance obtained above, it was shown that one amino group and 2 more carboxyl groups (3 free carboxyl groups in total) per molecule of eisenin became free

In order to confirm whether this partial hydrolysis product consists mainly of tripeptide or mixture of amino acids, or amino acid and dipeptide, and also to investigate the arrangement of the amino acids in the peptide, it was submitted to the oxidation by means of nitrous acid as well as hydrogen peroxide according to the methods used by Kendall, McKengie and Mason or by Quastel and Stewart for the study of glutathione.

On oxidation with nitrous acid, neither α -oxy-glutaric acid nor lactic acid was detected directly in the reaction product, indicating that there was no contamination with amino acid; after a complete hydrolysis, however, *dl*- α -oxy-glutaric acid, *dl*-glutamic acid and *dl*-alanine were isolated from the oxidised solution.

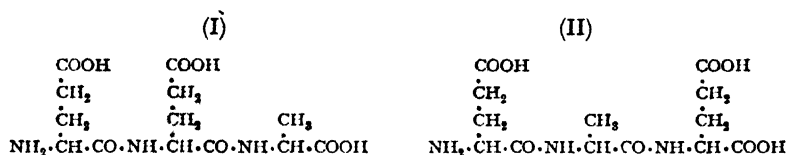
Therefore it is thought that one of the two glutamic acid molecules is attached through its carboxyl group and not through its amino group, also that the amino groups of another glutamic acid and alanine are substituted.

In the case of the reaction towards hydrogen peroxide, succinic acid and acetic acid were isolated by extracting the solution with ether directly after oxidation. From the solution exhausted with ether no more of these organic acids could be obtained though the solution was hydrolised with sulphuric acid.

This result shows that the glutamic acid, in this tripeptide molecule, is always attached to the amino group of the other amino acid by the carboxyl group which is next to the amino group.

But it is not possible to determine, by these reactions, whether only amino group or both amino and carboxyl groups in the alanine are concerned in the peptide connection.

From these results, it is concluded that the reaction product yielded by partial hydrolysis of eisenin may be a tripeptide which is denoted by either *di*-[α -amino- γ -carboxy-butyryl]-alanine (I) or [α -amino- γ -carboxy-butyryl]-alanyl-glutamic acid (II).



Work is now in progress on the structure of eisenin, the results of which will be shortly communicated.

Studies on the Yeasts Found in "Miso." (Supp. Contribution)

Part 4. Discussion and Conclusion.

(pp. 7~17)

By Masatoshi MOGI.

(The Brewing Laboratory of Noda Shoyu Co. Ltd., Noda-machi, Chiba-ken, Japan;
Received Dec. 11, 1939.)

The author has isolated 13 new strains of yeast from 20 samples of "Miso" produced by this company and in various districts of this country.

Morphological and physiological properties of the yeasts were investigated in detail and they were accordingly classified as follows:—

Saccharomyces miso ♂ nov. sp.

" " var. 1 nov. sp., nov. var.

" " var. 2 " " .

" *miso* ♀ nov. sp.

Zygosaccharomyces miso γ nov. sp.

" " var. 1 nov. sp., nov. var.

" " var. 2 " " .

Pseudohansenula miso nov. genus, nov. sp.

Pichia miso nov. sp.

Torulopsis miso ♂ nov. sp.

" *miso* ♀ " .

" *uvae* (Pollacei et Nannizzi) Lodder var. *miso* nov. var.

Pseudomycoderma miso nov. sp.

The author wishes to express his deep gratitude to Prof. emeritus Dr. T. Takahashi and Prof. Dr. K. Sakaguchi for their kind advice and encouragement throughout this work.

Biochemical Studies on the Sexual Organs of the Silk Worm, *Bombyx mori* L.

Part IV. On the quantitative development and the catalase actions
of the slimy gland, appendages of the female sexual organ.

(pp. 18~22)

By Takeo NAKASONE.

(Mie Prefectural Sericultural Experiment Station; Received Dec. 1, 1939.)

Studies on the Preparation of Unsaturated Higher Fatty Alcohol by the High Pressure Hydrogenation in the Presence of Zinc Catalyst.

Part III. On the Reduction of the Methylesters of the
Mixed Fatty Acids from Soya Bean Oil.

(pp. 23~26)

By Yuichi SHINOZAKI and Shizuo SUMI.

(Dept. of Organic Chemistry, The Central Laboratory, South Manchuria Railway Co.,
Dairen; Received Nov. 21, 1939.)

Nutritive Value of Cereals and Tubers.

(Studies on Rural Foods. I.)

(pp. 27~35)

Hisayoshi IWATA.

(Morioka Imperial College of Agriculture and Forestry, Japan;

Received Dec. 16, 1939.)

Various kinds of polished cereal grains, dried tubers and chestnuts powder were compared as to their food values by using them as basal diets in feeding experiments on young albino rats.

On the Reaction and Line Status of Apple Orchard Soil in South-Manchuria.

(pp. 36~38)

By R. KAWASHIMA.

(Agr. Chem. Laboratory, Kyushu Imp. University; Received Dec. 20, 1939.)

The author has determined both the reaction and degree of lime saturation of several apple orchard soils in Ryoto peninsula of South-Manchuria. The pH values of many soils now examined are slightly over 7 and the degrees of lime saturation are generally more than 80.

On the other hand, the apple orchard soils of Nagano and Aomori in Japan are acid in reaction almost unexceptionally, and lime saturation is low. As the varieties of apple cultivated are the same between Japan and Ryoto peninsula, a question arises which of these two opposing characters of soil conditions is most suitable for apples.

In the author's opinion, it is necessary to apply more lime for the apple orchard in Japan and make the soil less acid.

On the Retting of Vegetable Fibre Materials.

Part XI. The Useful Anaerobes for the Bacterial Retting of Flax.

(pp. 39~42)

By Tosio NAKAHAMA.

(Kanebo Yamashina Institute; Received Nov. 29, 1939.)

Nearly the same effective retting of flax was attained by the anaerobic process as was previously observed with the aerobic bacteria (see Part IX and X), and eleven strains of anaerobic bacteria were isolated from the retting vat.

After carrying out pure fermentation of flax with each of these eleven strains of bacteria, one strain of bacillus and one strain of coccus were selected as the most useful organisms.

One of the useful anaerobes was classified as a new species and named *Micrococcus linumus*, since the characteristics of the bacteria were found not to be the same as those of *Micrococcus minimus* Giselli, in the propagation on milk or potato and for the sources of nitrogen.

The other useful anaerobe was found to reveal similar characteristics to *Bacillus aurantius* Sack. However, cellulose was never decomposed and the saccharification of starch was not remarkable by the bacteria. For the fermentation products, acetone or butyric acid was not detected.

It was therefore concluded that this bacteria was also a new species and it was named *Bacillus linumus*.

On the Hydrolysis of Fats and Fatty Acid Esters. (V)

(pp. 43~54)

By Toyoki ONO.

(Chemical Laboratory of the Fish Meal Association of Japan;
Received Dec. 21, 1939)

I. Preparation of Triglycerides.

(A). Caprylic, capric, lauric, myristic, arachidic, erucic, ricinoleic, linolic, linolenic, $C_{21}H_{42}O_2$, and clupanodonic acid were isolated from cocoanut oil, peanut oil, rape oil, castor oil, linseed oil and sardine oil. Purified palmitic, stearic and oleic acid from commercial products.

(B). Simple triglycerides were obtained by Berthelot's method with these fatty acids—on heating for 5 hours at $120\sim 200^\circ C$ the mixture of glycerol, an excess of fatty acids and a small quantity of Twitchell's reagent.

II. Hydrolysis of Triglycerides by Pancreas Lipase.

(A). On the triglycerides of saturated fatty acids ($C_8\sim C_{18}$), the reaction velocity on hydrolysis diminishes in proportion to the molecular weight.

(B). On the triglycerides of unsaturated fatty acids (C_{18} and C_{22}), however, there is no relation between the reaction velocity and the molecular weight, but at lower temperature the reaction velocity depends upon the number of double bond (unsaturation) in glyceride.

III. Comparison of Hydrolysis between Oils and Glycerides.

Previous work showed that the saturated fats such as cocoanut oil, butter fat and beef tallow are much less hydrolysed at lower temperature than the unsaturated

ones such as perilla oil, whale oil and sardine oil.

From these experimental results it will be understood that the difference of reaction velocity, especially at lower temperature, depends upon the chemical composition of fat and oil—the contents of saturated or unsaturated glycerides. Table V. shows distinctly this explanation.

Table V. The Temperature Coefficient on Hydrolysis of Fats and Triglycerides.

| Fat or Oil | k/k' | Fat or Oil | k/k' | Triglyceride | k/k'' | Triglyceride | k/k'' |
|-------------|--------|-----------------|--------|--------------|---------|--------------|---------|
| Linseed oil | 7.45 | Castor oil | 3.46 | Caprylin | 8.14 | Olein | 8.14 |
| Perilla oil | 5.22 | Chicken fat | 7.81 | Caprin | 12.44 | Erucin | 9.80 |
| Olive oil | 6.93 | Whale oil | 3.51 | Laurin | 17.15 | Linolin | 7.02 |
| Beef tallow | 9.10 | Sardine oil | 5.96 | Myristin | 17.37 | Linolenin | 7.72 |
| Butter fat | 9.14 | Shark liver oil | 4.27 | Palmitin | 15.31 | Clupanodonin | 5.03 |
| Cocanut oil | 11.73 | Cod liver oil | 5.93 | Stearin | 9.36 | Ricinolein | 4.46 |

k , k' , k'' represent the reaction velocity coefficient at 30°, 0°, -10°C.

The Influence of Monochromatic Lights on the Action of Enzymes. [Report XXX~XXXIII]

Especially on the Influence of Infra-red Rays.

(pp. 55~63)

By Reitaro MURAKAMI.

(Agricultural College, Utunomiya; Received Dec. 22, 1939.)

In order to further investigate the influence of infra-red rays on the enzymes in yeast, the enzyme solutions containing saccharase, amylase, proteinase and lipase respectively were irradiated by infra-red rays from a "Vim Ray" red lamp. The treatments after the addition of the enzyme solutions into the substrates were the same as described in the author's previous papers.⁽¹⁾

In this experiment, the action of the yeast saccharase was found to be promoted by infra-red rays. The saccharase was more promoted by the rays containing both infra-red and visible.

The amylase, proteinase and lipase were influenced very slightly by the action of lights. However, the enzymes were promoted by infra-red rays and the rays containing both infra-red and visible.

(1) Bull. Agri. Chem. Soc. (Japan), 176, 435~444 (1939).

Phosphoric Acid Absorption of Soils in Tyosen. V.

(pp. 64~70)

By MISU-Hideo.

(Agricultural Experiment Station, Government General of Tyosen;

Received Aug. 28, 1939.)

Beiträge zur Kenntnis der Chemie des Muskeleiweißes.

I. Mitteilung. Über die Stickstoffverteilung des Kaninchenmuskelplasmas.

(ss. 71~81)

Von M. KADATSU.

(Aus dem Agrikulturchemischen Institut der Kaiserlichen Universität

Tokyo. Vorstand: Prof. Dr. E. Hiratsuka.)

(Eingegangen am 26. Dez. 1939.)

Zusammenfassung.

Mit etwa 2 kg. schweren männlichen Kaninchen wurden die folgenden Untersuchungen angestellt.

1) Nach einigen Versuchen mit der Muskulatur der hinteren Extremitäten wurde eine Methode angewandt, in der das Muskelplasma mittels Zentrifugalmaschine ungefähr quantitativ getrennt und bestimmt werden konnte. Dabei bestimmte der Verfasser das Prozent des getrennten Muskelplasmagewichtes zur ursprünglichen Muskulatur des Grades der Muskelplasmatrennbarkeit und deutete es als ein Zeichen von Muskelfleischzustandsänderung.

2) An fünf Lokalitäten der hinteren Extremitäten, an drei vom Rückgratsmuskel und an zwei von den Vordergliedern der drei oben erwähnten Kaninchen, die blutig durch Gnickschlag getötet wurden, ließ sich nach einer Probeentnahme nach einer Lagerung von 18, 48 beziehungsweise 118 Stunden im Eisschrank (0~4°C) der Muskelplasmatrennbarkeitsgrad und der Gesamt-, Rest-, Amino- (nach Folin), Ammoniak-stickstoffgehalt (nach Parnas-Heller) im Muskelplasma des Muskelfleisches derselben bestimmen.

3) Der Muskelplasmatrennbarkeitsgrad ändert sich nicht nur nach den Individuen, sondern auch der Muskellokalität und hat die Neigung, an beiden Muskelenden geringer als am Mittelteil zu sein. Seine Werte vergrößern sich in der Reihenfolge: Rückgrats-, hintere Extremitäten- und Vordergliedmuskeln; sie sind besonders groß an den hinteren Enden der ersteren.

4) Der Gesamt- und Eiweiß- (koagulierbare) Stickstoffgehalt des Muskelplasmas ist im allgemeinen geringer an beiden Enden des Muskels als am Mittelteil, diese Neigung äußert sich klar an den Rückgratsmuskeln, aber nicht an

den hinteren Extremitätenmuskeln. Zu dieser Tatsache wird der Zusammenhang von Muskelform und Tätigkeit erörtert.

5) Rest-, Amino- und Ammoniak-stickstoffgehalt zeigen dieselbe Neigung an den hinteren Extremitäten wie der Gesamtstickstoffgehalt; an den hinteren Enden des Rückgratsmuskels ist das Verhältnis jedoch ein umgekehrtes.

6) In der Stickstoffverteilung im Muskelplasma beträgt der Eiweißstickstoff an den hinteren Extremitäten 79~83% (Reststickstoff 17~21%) und variiert mehr und mehr vom Mittelteil nach den beiden Enden. Es ist, jedoch beachtenswert, daß der Reststickstoff an den hinteren Enden des Rückgratsmuskels 35~42% des Gesamtstickstoffes erreicht.

Amino- (5~7%), Ammoniak- (1~3%) stickstoffverteilung sind an den Lokalisationen groß, an denen der Reststickstoff groß ist.

7) Die Variierung der oben erwähnten Werte zwischen den Muskellokalitäten des hinteren Extremitätenmuskels ist am nächsten zu den experimentellen Fehlergrenzen, wenn dieselben auf beide Enden oder auf das hintere Ende entfallen, und an diesem Punkt läßt sich die Homogenität der hinteren Extremitätenmuskeln erkennen.

On the Formation of Ascorbic Acid from Mannose in Plants and in Animal Bodies. IV.

(pp. 82~83)

By Tetutarō TADOKORO and Tuneyuki SAITO.

(Hokkaido Imperial University; Received Dec. 16, 1939.)

On the Formation of Ascorbic Acid from Mannose in Plants and in Animal Bodies. V.

(pp. 84)

By Tetutarō TADOKORO.

(Hokkaido Imperial University; Received Dec. 16, 1939.)

The Effect of Glutathione upon Narcotism.

(Biochemical Studies on Glutathione. The IXth Report.)

(pp. 85~102)

By Masayoshi OGAWA.

(Department of Nutrition, College of Medicine, Nippon University;
Received Nov. 24, 1939.)

In this report the author described an experiment on the effect of glutathione

upon narcotism, employing a number of male albino rats weighing from 100 grams to 150 grams, which were narcotised by subcutaneous injections of bromral (90 mg per 100 grams of the body weight) and obtained the following results.

Rate of Sleep.

| The time of the inj. of GSH after the inj. of bromral. | GSH (mg) injected (per 100 grams of the body weight) | | | | | | | | |
|--|--|--------|--------|--------|--------|--------|---------|---------|---------|
| | 0 mg | 0.1 mg | 0.5 mg | 1.0 mg | 2.5 mg | 5.0 mg | 10.0 mg | 25.0 mg | 50.0 mg |
| 1 hr before the inj. of bromral. | 100 | 68 | 68 | 135 | 129 | 135 | 178 | 166 | 201 |
| At the same time. | 100 | 123 | 148 | 203 | 197 | 221 | 185 | 166 | 209 |
| Injected 1 hr after the inj. of bromral. | 100 | 123 | 197 | 203 | 172 | 166 | 215 | 184 | 191 |
| Inj. 2 hrs after the injection of bromral. | 100 | 116 | 178 | 240 | 227 | 209 | 233 | 178 | 203 |

As shown in the above table, the animals injected with bromral and GSH slept soundly and more deeply than the animals which were injected with bromral only.

By injecting a large dose such as 100 mg. of bromral per 100 grams of the body weight, and at the same time, injecting GSH in doses of 0 mg., 5 mg., 10 mg., 20 mg. and 30 mg. respectively per 100 grams of the body weight, the author obtained the following results:

Death or Recovery of the Animals.

| Body weight (gram) | Bromral (mg) injected | GSH (mg) injected | Doses of GSH (per 100 grams of the body weight) | Death | Recovery |
|--------------------|-----------------------|-------------------|---|-------|----------|
| 102 | 90 | 0 | 0 | + | - |
| 149 | 149 | 0 | 0 | + | - |
| 130 | 130 | 0 | 0 | + | - |
| 112 | 110 | 0 | 0 | + | - |
| 174 | 175 | 9 | 5 | + | - |
| 145 | 150 | 15 | 10 | + | - |
| 146 | 150 | 15 | 10 | + | - |
| 136 | 140 | 14 | 10 | + | - |
| 170 | 170 | 17 | 10 | + | - |
| 152 | 150 | 23 | 15 | - | + |
| 172 | 170 | 25 | 15 | - | + |
| 124 | 120 | 25 | 20 | - | + |
| 155 | 150 | 30 | 20 | - | + |
| 147 | 150 | 30 | 20 | - | + |

| | | | | | |
|-----|-----|----|----|---|---|
| 144 | 160 | 33 | 20 | + | - |
| 149 | 150 | 50 | 30 | - | + |
| 149 | 150 | 45 | 30 | - | + |
| 169 | 170 | 50 | 30 | - | + |

As shown in the above table, all the animals injected with 0 mg., 5 mg., or 10 mg. of GSH per 100 grams of the body weight died (death rate was 100%), but only 11% of the animals injected with 15 mg., 20 mg., or 30 mg. of GSH per 100 grams of the body weight died.

A Study on Bacteria of Korean Soy Preserved-Crabs.

(pp. 103~126)

By Y. L. Pak M. D.

(Seoul, Chosen (Korea); Received Oct. 28, 1939.)

This is report on the study of bacteria isolated from the various parts of the soy-preserved crab, the liver, generative organs, leg muscles. This long preserved crab is a favorite dish for Koreans.

A study was made on the bio-chemical nature, mode of growth, fermentative actions and also on the fermentation products of the bacteria isolated, the identities and varieties of which were as follows:—

1. *B. megatherium* var. K. S. C.
2. *B. mycoides* var. K. S. C.
3. *B. mesentericus* var. K. S. C. No. 1.
4. *B. mesentericus* var. K. S. C. No. 2.
5. *B. fusiformis* var. K. S. C. No. 1.
6. *B. fusiformis* var. K. S. C. No. 2.
7. *B. panis* var. K. S. C.
8. *B. lentus* var. K. S. C. No. 1.
9. *B. lentus* var. K. S. C. No. 2.
10. *B. spinosporus* var. K. S. C. No. 1.
11. *B. spinosporus* var. K. S. C. No. 2.
12. *B. agri* var. K. S. C.
13. *B. teres* var. K. S. C.
14. *B. simplex* var. K. S. C. No. 1.
15. *B. simplex* var. K. S. C. No. 2.
16. *Phytomonas fluccumfaciens* var. K. S. C. No. 1.

17. *Phytomonas flaccumfaciens* var. K. S. C. No. 2.

18. *Mic. epimetheus* var. K. S. C.

19. *Mic. aurantiacus* var. K. S. C.

Bulletin of the Agricultural Chemical Society of Japan.

TRANSACTIONS

Studies on Vitamin B₂ Complex. VI

Rat-acrodynia and Fatty Acids.*

By Ume TANGE.

(The Institute of Physical and Chemical Research.)

Received Nov. 13, 1939.

In 1932, I reported that when young rats were maintained on the diets totally deprived of fats they developed characteristic symptoms accompanying impairment of growth, denuded area of skin, and "scaly" condition of feet, which were curable by the administration of either linoleic or linolenic acid.⁽¹⁾ Later, in the studies on vitamin B₂ deficiencies^(2,3), it was found that rats suffering from lack of vitamin B₂ often developed symptoms similar to those of the fat deficiency mentioned above. However, when the diets contained moderate amounts of fats, for instance 10%, the symptoms were irregular and not so severe as with fat free diets. Birch and Gyorgy⁽⁴⁾ reported that certain fats had a sparing action on vitamin B₂, and they suggested that this action was due to the linoleic acid present in the fat. Salmon⁽⁵⁾ showed that oils alone or starch alone failed to cure or prevent acrodynia and that oils did not contain the entire dermatitis-preventing factor, but might contain an essential part of the factor which supplemented the heated yeast extract. More recently Birch⁽⁶⁾ reported that two factors were concerned in the cure of the acrodynia-like dermatitis. One was the water-soluble factor vitamin B₂; the other was fat-soluble and present in the fatty acid fraction of maize oil, which appeared to be similar to the "linoleic acid" of Burr and Burr.

The experiments presented in this paper are, therefore, concerned with the relation between vitamin B₂ and unsaturated fatty acids in the cure and production of acrodynia-like dermatitis of rats.

EXPERIMENTAL.

Methods.

In order to carry out this experiment, it was needed to prepare pure casein and vitamin B₂ extract free from fats. The following procedure, therefore, was adopted.

Purification of casein.— 2 kg of casein was stirred into 5 litres of water solution of 80 g of NaCl containing 6 cc of glacial acetic acid. After settling for several hours, the supernatant liquid was decanted off, and a similar treatment was repeated six times more. This was filtered on a large Buchner's funnel, washed free from acid, and then the casein was stirred into 4 litres of 95% alcohol. The alcohol was removed by filtration. This procedure was repeated once more, the casein was dried at about 50°C, and ground, then extracted with ether for 10 days to remove fatty materials completely.

Preparation of yeast extract.— 200 g of dried brewer's yeast was extracted with 800 cc of 75% alcohol by shaking for 2 hours at room temperature. It was filtered and reextracted as above, the process being repeated twice more. The combined extracts were then evaporated down to remove the alcohol, adjusted to pH 2 with HCl, and shaken with ether several times to remove all neutral fats and fatty acids. The solution was made to about pH 6 with NaOH, and concentrated in vacuo. 95% alcohol was added into the concentrate and the mixture was allowed to stand in the ice box until the inert materials had settled out, which were filtered off and the filtrate kept for assay (10 drops of this solution correspond to the yield from 0.8 g of the dried yeast).

A. Estimation of vitamin B₆ activity of the yeast extract. The basal diet used in this experiment had the following composition:

Diet I.

| | |
|--|-----|
| Purified casein | 18% |
| Sucrose (<i>Pharmacopeia Japonica</i>) | 67 |
| Butter fat | 9 |
| McCullum's salt mixture | 4 |
| Agar-agar | 2 |

This was supplemented with 10 γ B₁ hydrochloride, 20 γ riboflavin each rat daily, and 2 drops of biosterin** (45,000 I. U.) weekly as vitamins A and D.

The feeding technique employed in this work was for the most part similar to that described in the preceding paper.⁽⁷⁾

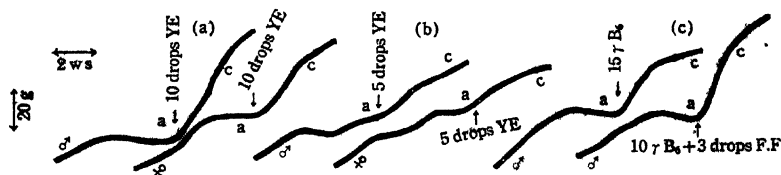


Fig. 1. Growth curves of rats on Diet I, supplemented daily by 5 or 10 drops of yeast extract, and B₆ alone or combination of B₆ and FF (filtrate factor), respectively. a acrodynia, c cured, FF (one drop=1.5 g of fresh beef liver).

From Fig. 1 (a and b), it may be noticed that the cure of acrodynia and

the response of growth were slow with 5 drops of the yeast extract, while more rapid cure and growth resulted with 10 drops of the extract. With 15 γ of crystalline B₆,*** nearly the same result as with 10 drops of the yeast extract was obtained. By supplementing vitamin B₆ with 3 drops of filtrate factor, however, a very striking effect on growth was brought about as seen from Fig. 1 (c). This was in agreement with the previous experiments.⁽³⁾

Filtrate factor used throughout the experiments was prepared from beef liver by the method previously described.⁽³⁾

B. In consideration of the results obtained in the previous experiments⁽¹⁾⁽²⁾, the following attempts were made; to study firstly, the influence of vitamin B₆ on the production of the fat-deficient disease, and secondly, the influence of fats in vitamin B₆-free diets on the development of the acrodynia-like dermatitis. The basal diets used in these investigations are shown in Table I.

1. Experiments on fat-free diet. This was carried out by feeding rats on diets provided with different amounts of vitamin B₆. The basal fat-free diet is given in Table I.

TABLE I.
Composition of diets (per cent.)

| Component | Diet | Diet II | Diet III | Diet IV |
|--|------|---------|----------|---------|
| Purified casein | | 20 | 20 | 20 |
| Sucrose (<i>Pharmacopeia Japonica</i>) | | 73 | 70 | 70 |
| McCullum's salt mixture | | 5 | 5 | 5 |
| Agar-agar | | 2 | 2 | 2 |
| Soy bean oil | | — | 3 | — |
| Crisco | | — | — | 3 |

All the above diets were supplemented as in Diet I

Group 1. The rats receiving 10 drops of the yeast extract grew well for 5 to 7 weeks, then the growth was retarded. At about 9 to 10 weeks the nose and mouth were inflamed, and scaly feet, dandruff and alopecia appeared, but no typical acrodynia was noticed. When this condition continued the rapid loss in weight occurred and death happened shortly unless fatty acid was fed. Administration daily of 5 to 10 drops of soy bean oil or 2 drops of linoleic acid brought about increase in weight and rapid cure, but crisco had apparently no such curing properties (Fig. 2).

Linoleic acid was prepared from linol-hydroxamic acid† ($C_{17}H_{31}-C \begin{smallmatrix} \nearrow \text{NHO} \\ \searrow \text{OH} \end{smallmatrix}$)—.

10 g of pure linol-hydroxamic acid (mp 41~42°C), which was separated from

cotton-seed oil, was added into a mixture of 100 g of 70% ethyl alcohol and 8 g of H_2SO_4 , and it was heated on a water bath under reflux condenser in the atmosphere of CO_2 until no more purple red colour reaction with $FeCl_3$ appeared; it took about three hours. The resulting solution was distilled in a reduced pressure to remove the alcohol, adding a small amount of water from time to time. The solution was now extracted with petroleum ether below bp $50^\circ C$, the extraction being repeated twice more. After removing the ether by distillation, the residual solution was saponified with alcoholic potassium hydroxide in order to remove the ethyl ester which might be present. This alkaline solution was now acidified with HCl , and again extracted with petroleum ether. After dehydrating with anhydrous Na_2SO_4 , the petroleum ether solution was evaporated as completely as possible in a high vacuum in CO_2 atmosphere. The yield of linoleic acid was nearly theoretical.

Group 2. The animals given 5 drops of the yeast extract grew for the first few weeks, but gradually declined in weight. They developed acrodynia-like dermatitis within 5 to 7 weeks, accompanying scurfy coat and denuded area on the skin. With 5 drops of soy bean oil, the improvement in weight and dermatitis was much slower than with 10 drops of the oil. Feeding 2 drops of linoleic acid brought about a prompt cure of dermatitis, whereas crisco was ineffective (Fig. 3).

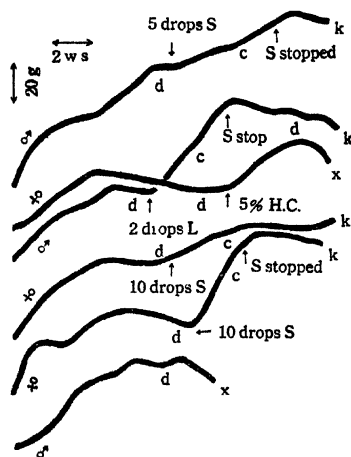


Fig. 2. Growth curves of rats on fat-free diet (Diet II), supplemented daily by 10 drops of yeast extract.

d; fat-deficiency, S; soy bean oil, L; linoleic acid, H. C.; crisco, k; killed, c; cured, x; died.

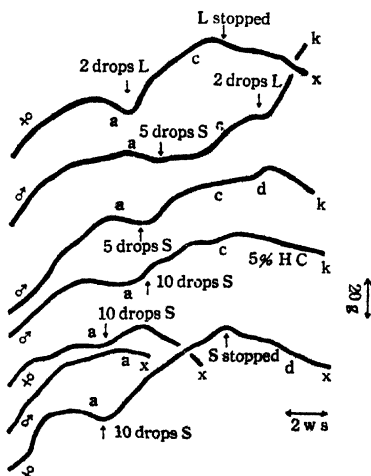


Fig. 3. Growth curves of rats on fat-free diet (Diet II), supplemented daily by 5 drops of yeast extract.

a; acrodynia, d; fat-deficiency. The other abbreviations are the same as in Fig. 2.

These results indicate that rats may develop the acrodynia-like dermatitis if diet is free from fat even when moderately large amounts of vitamin B_6 are given. It would seem, therefore, that certain fats are necessary for normal growth of rats,

2. Experiments on vitamin B₆-free diets. This was carried out by feeding animals on diets containing varying amounts of fats to determine the time of the development of acrodynia-like dermatitis and the degree of the symptom. The diets used are shown in Table I.

Group 1. The rats fed on Diet II (fat-free diet) developed severe acrodynia in 3 to 4 weeks. Administration of 10 drops of the yeast extract caused some improvement on the symptom, but the rats declined in weight and death occurred among them. By the additional supplement, however, of 10 drops of soy bean oil or 2 drops of linoleic acid, there was an immediate resumption of growth and the symptoms cleared up within a few weeks. Cured animals maintained themselves free of all symptoms as long as the fatty acid and yeast extract were continued. If the oil or fatty acid was withheld, the rats declined in weight and developed acrodynia (Fig. 4).

Group 2. The acrodynia-like dermatitis appeared in about 6 to 7 weeks in the rats fed with Diet III (3% soy bean oil). By providing 5 or 10 drops of the yeast extract the acrodynia was quickly cured and growth restored. When 15 γ of B₆ was given the dermatitis was cured, but filtrate factor was needed to induce optimum growth in the rats. Soy bean oil alone did not prevent the acrodynia but did delay the onset of the symptom to some extent (Fig. 5).

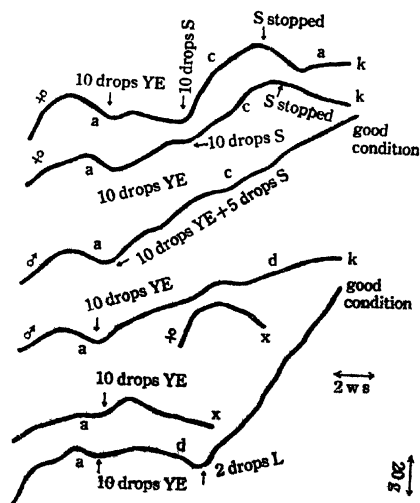


Fig. 4. Growth curves of rats on vitamin B₆-free diet containing no fat, YE; yeast extract. The other abbreviations are the same as in Figs. 2 and 3.

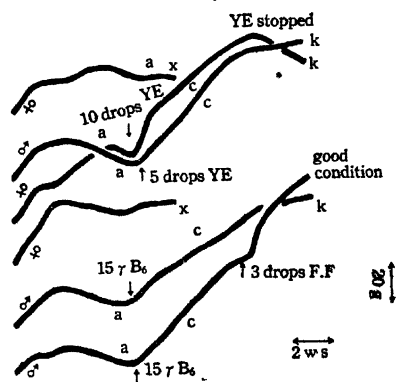


Fig. 5. Growth curves of rats on vitamin B₆-free diet containing 3% soy bean oil, YF; filtrate factor. The other abbreviations are the same as in Figs. 3 and 4.

Group 3. Results obtained with Diet IV (3% crisco) were similar to those with Diet II (fat-free) except the delayed onset of the dermatitis, which was not

so severe as seen in the animals on Diet II. Without supplement of the yeast extract, the animals died with rapid loss of weight. 5 drops of the yeast extract

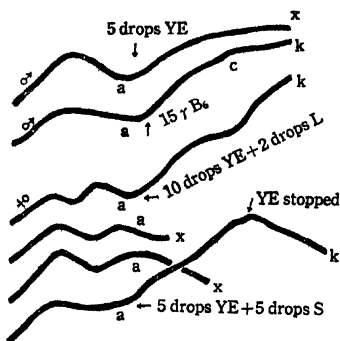


Fig. 6. Growth curves of rats on vitamin B_6 -free diet containing 3% crisco.

The abbreviations are the same as in Figs. 3 and 4

nia develops very quickly, with subnormal weight and scaliness of hind feet. Feeding the yeast extract relieves the symptoms to some extent, but for complete cure and normal growth it is necessary also to administer certain fatty acids. It is seen that the effect of soy bean oil on cure of the dermatitis and promotion of the growth depends on its content of the essential fatty acid, which may perhaps be similar to linoleic acid.

5 drops of the extract gave a slow improvement. Complete cure and normal growth were obtained by an additional supplement of either 5 drops of soy bean oil or 2 drops of linoleic acid (Fig. 6).

The results with 10% crisco diet were not shown in the figures but were similar to those observed with the 3% crisco diet. The development of acrodynia was irregular, and the onset was delayed, usually requiring 10 to 12 weeks.

These results show that when rats are fed on the basal fat-free diet without any addition of vitamin B_6 , severe acrodynia

Discussion.

The evidence indicated that two factors were concerned in the cure and production of acrodynia like dermatitis; one water-soluble factor, *viz.*, vitamin B_6 , and the other fat-soluble factor apparently similar to linoleic acid. On examining the results it was at once recognized that the close relationship between the amount of certain fatty acids in the diet and severity of the acrodynia-like dermatitis would indicate that vitamin B_6 was connected in some way with the metabolism of the fatty acids. Though the exact biological function between vitamin B_6 and the essential fatty acid has not yet been fully established, it seemed reasonable from the results to conclude that the presence of an adequate amount of vitamin B_6 and of the essential fatty acid in diet was necessary for normal health and growth of the animal. Birch⁽⁶⁾ found the unsaturated fatty acids of maize oil to be effective in relieving the symptoms of vitamin B_6 deficiency, and suggested this finding to be related to the observations of Burr and Burr⁽⁷⁾ concerning essential fatty acids, and perhaps also to the fat-soluble antidermatitis factor indicated by Hogan and Richardson.⁽¹⁰⁾ He could not find any evidence to indicate that the vitamin might exist in combination with lipoids, but he presented, instead, the evidence showing that there was a functional relationship between the unsaturated

fatty acids and the vitamin. Halliday⁽¹¹⁾ reported further evidence supporting this view, who observed that there was fatty liver in vitamin B₆-deficient animals and feeding choline remedied such condition to a large extent. Quackenbush and Steenbock⁽¹²⁾ found that a B₆-deficient diet supplemented by unsaturated fatty acids, either as natural oils or as 10 mg per day of ethyl linoleate, protected rats from acrodynia and kept them in good health. Salmon⁽⁷⁾ also observed a relation between B₆ and fat metabolism. Such reports led to the conclusion that vitamin B₆ was connected in some way to fat metabolism.

SUMMARY.

Data are submitted which show that two factors are concerned in the production and cure of the acrodynia-like dermatitis. One is water-soluble factor, *viz.*, vitamin B₆; the other is fatty acid factor similar to linoleic acid.

The evidence suggests that vitamin B₆ is connected in some way with the metabolism of the fatty acids.

I wish to thank Professor U. Suzuki for his many helpful suggestions concerning this work. I am also indebted to Misses M. Takahashi and H. Sasaki for their generous assistance in preparing the materials and feeding the animals.

* This paper was presented at the Scientific Meeting of I. P. C. R. June 16, 1939.

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(5) W. D. Salmon: J. Biol. Chem., **123**, CIV, (1938).

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** I desire to thank Mr. Kasama for supplying biosterin.

(7) U. Tange: Sc. Pap. I. P. C. R., **36**, 471, (1939).

*** I Wish to thank Mr. A. Ichiba and Miss K. Michi for the generous gift of crystalline B₆.

(8) U. Tange: Sc. Pap. I. P. C. R., **35**, 47, (1938).

† I wish to express my great indebtedness to Dr. Y. Inouye for providing the linol-hydroxamic acid.

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ABSTRACTS

from

TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

A Brown Forest Soil in Kokuga of North-Manchuria.

(pp. 125~128)

A. KAWASHIMA and G. SUYAMA

(Agr. Chem. Laboratory, Kyushu Imp. University; Received Jan. 20, 1940.)

Kokuga is situated on the river Amur in lat. $50^{\circ} 15' N.$ and long. $127^{\circ} 29' E.$ The soil profile now concerned exhibits clear morphological characteristics of a slightly podzolized brown forest soil influenced by soil water to some extent. The data described below are all expressed on air-dry basis.

Some analytical data on fine soil are given in Table I. The exchange capacity and exchangeable calcium are expressed as mg. eq. per 100 g. soil.

Table I. Some analytical data on fine soil.

| Layer | Moisture % | Loss on ignition % | Total N % | pH | | Daikuhara acidity ($y_1 \times 3$) | Hydrolytic acidity (y_1) | Exchange capacity | Exchangeable Ca | % of Ca |
|----------------|------------|--------------------|-----------|------------------|------|--------------------------------------|------------------------------|-------------------|-----------------|---------|
| | | | | H ₂ O | KCl | | | | | |
| A ₁ | 7.34 | 10.85 | 0.36 | 5.33 | 4.27 | 3.9 | 32.1 | 36.59 | 16.43 | 44.9 |
| A ₂ | 6.11 | 6.07 | 0.10 | 5.59 | 4.24 | 5.7 | 21.8 | 27.70 | 13.66 | 49.3 |
| B ₁ | 6.98 | 4.94 | 0.09 | 5.66 | 4.46 | 3.6 | 15.8 | 28.72 | 14.79 | 51.5 |

As may be seen in Table I. in A₁-layer total nitrogen content and exchange capacity are very high and the pH-value and percentage saturation of calcium are relatively low.

The colloidal clays ($<0.001 \text{ mm } \phi$) were separated and analysed. The total contents of silica and sesquioxides and their molecular ratios are given in Table II, in which the loss on ignition and exchange capacity are also included.

Table II. Some analytical data on colloidal clay.

| Layer | Moisture % | Loss on ignition % | Exchange capacity (m. eq.) | SiO ₂ % | Al ₂ O ₃ % | Fe ₂ O ₃ % | $\frac{\text{SiO}_2}{\text{Al}_2\text{O}_3}$ | $\frac{\text{SiO}_2}{\text{Fe}_2\text{O}_3}$ | $\frac{\text{Fe}_2\text{O}_3}{\text{Al}_2\text{O}_3}$ |
|----------------|------------|--------------------|----------------------------|--------------------|----------------------------------|----------------------------------|--|--|---|
| A ₁ | 4.73 | 23.28 | 86.68 | 37.87 | 17.15 | 7.00 | 3.74 | 2.97 | 0.26 |
| A ₂ | 9.92 | 12.24 | 72.85 | 42.20 | 20.23 | 8.44 | 3.54 | 2.79 | 0.27 |
| B ₁ | 6.10 | 13.22 | 70.99 | 44.36 | 21.52 | 8.25 | 3.49 | 2.81 | 0.25 |

The high loss on ignition and exchange capacity in A_1 are due to the presence of some humus. The silica-alumina and silica-sesquioxide ratios in A_1 are the greatest, and that means some leaching down of colloidal sesquioxides from this layer. But as the differences in the magnitude of these ratios between each layer are very insignificant, a fairly good similarity of composition between these colloidal clays can be assumed.

Phosphoric Acid Absorbtion of Soils in Tyosen. (VI~VII)

(pp. 129~144)

By MISU-Hideo.

(Agricultural Experiment Station, Government General of Tyosen ;

Received Aug 28, 1939.)

On the Enzymic Action of Nucleotid-like Substances. (II)

(pp. 145~146)

By Tetsutarō TADOKORO & Tsuneyuki SAITO.

(Hokkaido Imperial University ; Received Dec. 26, 1939.)

On the Hydrolysis of Fats and Fatty Acid Esters. (VI)

(pp. 147~158)

By Toyoki Ono.

(Chemical Laboratory of the Fish Meal Association of Japan ; Received Jan. 23, 1940.)

(I). Hydrolysis of Triglycerides by Ricinus Lipase.

Triglycerides are less attacked by ricinus lipase than by pancreas lipase, especially the hydrolysis of triricinolein took place with the smallest velocity. On the contrary, the glycerides consisting of the same ricinoleic acid, castor oil, is split rapidly.

These facts seemed to be due to the difference in the emulsification of the substrates.

(II). Hydrolysis of Esters by Pancreas and Ricinus Lipase.

(A). Forty-six esters of organic acids were prepared in this laboratory by Haller's method with acids (aliphatic and aromatic acids) and alcohols (methyl, normal and iso-propyl, normal and iso-butyl, amyl alcohols).

(B). The increase of the number of carbon atoms in alkyl group decreases

the rate of the hydrolysis of esters, and methyl esters of fatty acids are hydrolysed more easily than alkyl esters.

(C) The hydrolysis of esters has no such relation to the number of carbon atoms in fatty acid as in the case of the hydrolysis of triglyceride, except in the following system.



(D). Esters of unsaturated fatty acids are more rapidly attacked than saturated fatty acid esters with the same carbon atoms.

(E). Methyl and ethyl esters of formic, acetic, valeric, benzoic, salicylic and phthalic acid are hardly hydrolysed by pancreas and ricinus lipase.

(F). The differences in the hydrolysis of normalbutyl and isobutyl alcohol esters of fatty acids are not due to the different structures of the alcohols, but to the density of esters. Those between normalpropyl and isopropyl alcohol esters may be, however, attributed entirely to the structure of the alcohols.

Studies on the Absorption Spectra of Wheat Glutenin.

(pp. 159~162)

By Kinsuke KONDO and Hisateru MITSUTA.

(Nutritional Chemical Laboratory, Faculty of Agriculture, and Chemical Institute,
Kyoto Imperial University; Received Jan. 12, 1940.)

On the Carbohydrate in Wheat Gliadin.

(pp. 163~174)

By Kinsuke KONDO and Uichiro SARATA.

(Nutritional Chemical Laboratory, Faculty of Agriculture, and Chemical Institute,
Kyoto Imperial University; Received Jan. 12, 1940.)

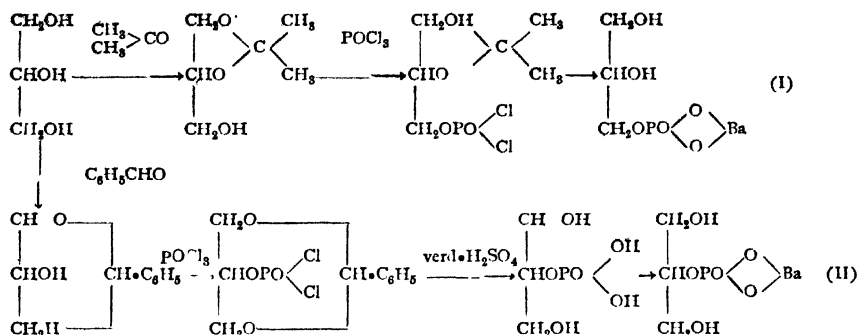
Die Synthese von α - und β -Glycerophosphorsäure.

(SS. 175~180)

Von Yataro OBATA.

(Landwirtschaftliches biochemisches Laboratorium der Kaiserlichen Universität
zu Tokyo; Eigegangen am 31. Jan. 1940.)

Der Verfasser synthetisierte α - (I) und β -Glycerophosphat (II) folgenderweise, um die Trennungsmethode der Isomeren von der Glycerophosphorsäure nach Karrer und Salomon¹⁾ nachzuprüfen:



Nach Perjodsäureoxydation²⁾ wurden die Produkte auf Abwesenheit der Acylwanderung³⁾ geprüft, und mit diesen geprüften Proben wurde ein Versuch über die Entstehung des schwerlöslichen Doppelsalzes mit Ba (NO₃)₂ gemacht. Wie schon von Karrer und Benz⁴⁾ gezeigt gestaltete das α -Ba-Salz nicht das schwerlösliche Doppelsalz aber trotz des Einspruchs von Kay⁵⁾ gestaltete das β -Ba-Salz das schwerlösliche Doppelsalz. Dieses Ergebnis stützt die Methode von Karrer und Salomon.¹⁾

Das α -Isomer, eine Glykolverbindung, ist oxydiert von Pb (IV)-Acetat⁶⁾ oder HIO₄.²⁾ Diese zwei Oxydationsbestimmungen wurden versucht in Vergleichung mit fast gleichem Resultate. Die Oxydation mit Pb (IV)-Acetat bedurfte längerer Zeit (20 Stdn.) und die Reagenz ist instabil. Im Gegensatz bedurfte die Oxydation mit Perjodsäure nur weniger Minuten (15 Min.) und α -Ba-Salz, ist deshalb viel nützlicher. Die Substanz ist hergestellt vom käuflichen Ca-Salz (Merck) nach der Methode von Karrer und Salomon.¹⁾

Pb (IV)-Acetat-Oxydation :

| Subst. (g) | Na ₂ S ₂ O ₃ (0.097872 N) (cc) gef. | Blindversuch | Oxydationswert (%) |
|------------|--|--------------|--------------------|
| 0.1020 | 8.4 | 12.7 | 87.55 |
| 0.0465 | 10.8 | " | 91.82 |
| 0.0629 | 9.8 | " | 92.52 |
| 0.0443 | 10.7 | " | 85.41 |
| 0.0587 | 10.06 | " | 85.46 |
| 0.0459 | 10.69 | " | 83.24 |
| | | | Mittelwert 87.73 |

HIO₄-Oxydation

| Subst. | Na ₂ S ₂ O ₃ (0.09834 N) (cc) gef. | Blindversuch | Oxydationswert (%) |
|--------|---|--------------|--------------------|
| 0.0250 | 11.8 | 14.7 | 87.63 |
| 0.0250 | 12.0 | 14.9 | 87.63 |

HIO₄-Oxydation des α -Ba-Salzes, synthetisch hergestellt von Acetonglycerin nach E. Fischer und Pfahler⁷⁾:

| Subst. | Na ₂ S ₂ O ₃ (0.09834 N) gef. | Blindversuch | Oxydationswert (%) |
|---------|--|--------------|--------------------|
| 0.025 g | 11.8 cc | 14.8 cc | 90.72 |

Die Synthese von β -Glycerophosphorsäure wurde durchgeführt vom Material 1,3-Benzylidenglycerin wie die Synthese von α -Ba-Salz von Acetonglycerin.

Das 1,3-Benzylidenglycerin ist oft schwer kristallisierbar, wenn man es nach Hibbert und Carter⁹⁾ herstellen will. Ich hatte ein gutes Resultat, wenn ich das ohne Reaktion bleibende Benzaldehyd wie Bisulfit ausnahm. Es war mir gelungen durch Schüttenln von Benzollosung der Produkte mit gesättigter Lösung von NaHSO_3 .

20 g von 1,3-Benzylidenglycerin (Fp 83.5~84 aus Aether; C 66.72 (66.66), H 6.68 (6.66)) und 15 g von POCl_3 werden zur Reaktion gebracht und der Zusammenhang zwischen der Reaktionsdauer und der Ausbeute an β -Ba-Salz ist wie folgt:

| Reaktionsdauer | Ausbeute | |
|----------------|----------|----------|
| 1 Std. | 3.5 g | (10 %) |
| 2 | 9.5 | (28) |
| 4 | 4.0 | (11.7'') |
| 10 | 2.6 | (7.6'') |
| 24 | 0 | (—) |

Oxydation von β -Ba-Salz mit HIO_4 :

| Subst. | $\text{Na}_2\text{S}_2\text{O}_3$ (0.09834 N) gef. | Blindversuch | Oxydationswert |
|---------|---|--------------|----------------|
| 0.025 g | 14.5 cc | 14.6 cc | 3.02% |
| 0.025 g | 14.6 | 14.7 | 3.02 |

Die Reaktion mit $\text{Ba}(\text{NO}_3)_2$: 2.5 g der Probe wurden in 50 cc Wasser gelöst, zu 30 cm eingengt und mit einer Lösung von 2.5 g $\text{Ba}(\text{NO}_3)_2$ in 50 cm Wasser gemischt.

Bei α -Ba-Salz gab es lange keine Veränderung; bei β -Ba-Salz gab es sofort eine weisse Trübung und nach 48 Stdn. wurden 2.44 g Prazipitat erhalten. Die Ausbeute ist 3.08 g (86%), wenn man 0.64 g (Löslichkeit 0.8%) gelöst zur Lösung hinzufügt. Die Analyse des Produktes: Ba 47.36% (47.04, P 7.12% (7.07).

Bei dem Gemisch von gleichen Mengen von α - und β -Ba-Salzen erreicht die Ausbeute an Doppelsalz 93%.

Diese Untersuchungen wurden von mir unter der Leitung des Herrn Prof. Bunsuke Suzuki ausgeführt, dem ich hiermit für seine Unterstützung meinen besten Dank ausspreche.

(Gelesen in der monatlichen Versammlung der Agrikulturchemischen Gesellschaft, Nov. 1938).

LITERATUR.

- (1) Karrer u. Salomon: *Helv.* **9**, 3 (1926).
- (2) Fleury et Paris: *C. r.* **196**, 1416 (1933).
- (3) E. Fischer: *B.* **53**, 1621 (1926).
- (4) Karrer u. Benz: *Helv.* **10**, 87 (1927).
- (5) Key: *J. Biol. Chem.* **91**, 135 (1931); *do.* **93**, 409 (1931); *Biochem. J.* **23**, 143 (1934).
- (6) Carrara: *Giorn. Chem. Ind. Appl.* **14**, 236 (1932).
- (7) E. Fischer u. Pfähler: *B.* **53**, 1589, 1606 (1929).
- (8) Hibbert u. Carter: *J. Am. C. S.* **51**, 1601 (1929).

(Während die Korrektur dieser Abhandlung fand ich den Bericht von Brägl u. Müller (*B.* **72**, 2126; 1939)).

Bulletin of the Agricultural Chemical Society of Japan.

TRANSACTIONS

Chemische Zusammensetzungen einiger Pflanzen in Taiwan.

Von T. HIRATA, K. HONDA, Y. NAKAMURA
und K. YAMAJI.

(Aus dem Institut für Zuckerforschung in Japan)

Eingegangen am 15. Feb. 1940

Um geeignete Materialien für die Herstellung der Zellstoffe zu wählen, haben wir in der vorliegenden Arbeit die chemischen Bestandteile des Zuckerrohrs, der Leguminosepflanzen und einiger anderer Pflanzen untersucht.

1. ZUCKERROHR.

Der Zuckerrohrstengel wurde in vier Portionen geteilt: A Knotenportion; B äußere Portion des Stengels, die Portionen A, B und D ausgenommen; und D Markportion.

Diese Portionen wurden getrennt mit Wasser gut gewaschen, in einem Morser zerrieben und dann in fließendem Wasser eingetaucht gelassen. Nach vollständiger Entfernung des Zuckers, wurde die Bagasse getrocknet und wieder pulverisiert.

Die Prozentsätze der so hergestellten Portionen sind folgende (Tabelle I):

Tabelle I.

| Portion | Rasse des Zuckerrohrs | | |
|---------|-----------------------|----------|----------|
| | 2725 POJ | 2878 POJ | 2883 POJ |
| A | 41,8 | 26,1 | 31,7 |
| B | 13,6 | 18,2 | 16,0 |
| C | 40,7 | 49,3 | 46,1 |
| D | 3,9 | 6,4 | 6,2 |

Die Analyse der Bagasse wurde im wesentlichen nach der Methode von Schorger ausgeführt (Tabelle II bis VI).

Tabelle II. 2725 POJ.

| Portion | A | B | C | D |
|--------------------------|-------|-------|-------|-------|
| In % der Trockensubstanz | | | | |
| Aether-Extrakt | 0,76 | 0,36 | 0,82 | 1,14 |
| Alkohol-Extrakt | 7,27 | 2,28 | 15,39 | 24,17 |
| Verd. Alkali-Extrakt | 37,78 | 28,75 | 47,31 | 53,89 |
| Heißes Wasser-Extrakt | 7,58 | 1,56 | 15,92 | 25,90 |
| Cellulose | 45,09 | 54,75 | 45,66 | 39,43 |
| Lignin | 22,56 | 21,93 | 17,43 | 12,97 |
| Pentosan | 29,94 | 28,28 | 26,93 | 21,46 |
| Stickstoff | 0,25 | 0,25 | 0,25 | 0,21 |
| Asche | 1,67 | 0,70 | 1,73 | 2,82 |

Tabelle III. 2878 POJ.

| Portion | A | B | C | D |
|--------------------------|-------|-------|-------|-------|
| In % der Trockensubstanz | | | | |
| Aether-Extrakt | 0,54 | 0,56 | 0,57 | 0,52 |
| Alkohol-Extrakt | 3,84 | 2,11 | 15,65 | 9,71 |
| Verd. Alkali-Extrakt | 34,33 | 22,09 | 45,91 | 40,83 |
| Heißes Wasser-Extrakt | 4,82 | 3,24 | 17,84 | 11,31 |
| Cellulose | 53,43 | 61,51 | 46,07 | 50,94 |
| Lignin | 23,92 | 22,81 | 17,05 | 13,52 |
| Pentosan | 25,67 | 25,98 | 23,48 | 22,07 |
| Stickstoff | 0,32 | 0,25 | 0,23 | 0,29 |
| Asche | 1,56 | 0,85 | 2,45 | 2,45 |

Tabelle IV. 2883 POJ.

| Portion | A | B | C | D |
|--------------------------|-------|-------|-------|-------|
| In % der Trockensubstanz | | | | |
| Aether-Extrakt | 0,71 | 0,95 | 0,63 | 1,02 |
| Alkohol-Extrakt | 3,64 | 4,37 | 9,21 | 23,03 |
| Verd. Alkali-Extrakt | 32,65 | 28,55 | 40,80 | 42,99 |
| Heißes Wasser-Extrakt | 5,89 | 5,63 | 10,98 | 22,69 |
| Cellulose | 52,79 | 58,33 | 50,17 | 42,27 |
| Lignin | 21,96 | 21,76 | 18,59 | 9,07 |
| Pentosan | 27,30 | 26,65 | 25,62 | 20,79 |
| Stickstoff | 0,30 | 0,26 | 0,24 | 0,29 |
| Asche | 1,68 | 0,93 | 1,43 | 2,09 |

Tabelle V. F 108.

| Portion | A | B | C | D |
|------------------------|--------------------------|-------|-------|-------|
| | In % der Trockensubstanz | | | |
| Asche | 1,45 | 1,11 | 1,74 | 5,12 |
| Heißes Wasser-Extrakt | 13,18 | 9,65 | 6,59 | 16,34 |
| Verd. Alkali-Extrakt | 32,92 | 28,72 | 44,28 | 54,68 |
| Alkohol-Benzol-Extrakt | 3,74 | 3,38 | 5,00 | 6,78 |
| Pentosan | 28,99 | 26,56 | 29,19 | 23,59 |
| Lignin | 24,60 | 22,21 | 22,19 | 15,97 |
| Cellulose | 48,32 | 56,06 | 45,97 | 36,85 |
| In % der Cellulose | α -Cellulose | 75,50 | 78,20 | 72,22 |
| | β -Cellulose | 17,64 | 13,92 | 13,99 |
| | γ -Cellulose | 6,86 | 7,88 | 13,79 |

Tabelle VI. Glagah, Kassoer und Chunnee.

| | Glagah | Kassoer | Chunnee |
|------------------------|--------------------------|---------|---------|
| | In % der Trockensubstanz | | |
| Asche | 1,12 | 1,31 | 1,53 |
| Heißes Wasser-Extrakt | 8,78 | 10,28 | 19,82 |
| Verd. Alkali-Extrakt | 27,60 | 30,71 | 46,25 |
| Alkohol-Benzol-Extrakt | 2,67 | 3,02 | 4,76 |
| Pentosan | 27,72 | 26,31 | 27,22 |
| Lignin | 29,63 | 27,40 | 21,45 |
| Cellulose | 64,22 | 62,76 | 47,29 |
| In % der Cellulose | α -Cellulose | 77,31 | 72,13 |
| | β -Cellulose | Spur | Spur |
| | γ -Cellulose | 22,69 | 27,87 |

Die Bagasse wurde in üblicher Weise mit Kaliumchromat und Salpetersäure behandelt und Länge und Breite der Faser bestimmt (Tabelle VII).

Tabelle VII.

| | | Äußerer Teil des Stengels | | | Innerer Teil des Stengels | | |
|---------|--------------|---------------------------|---------|--------|---------------------------|---------|--------|
| | | längst | kürzest | Mittel | längst | kürzest | Mittel |
| F 108 | Länge in mm | 3,680 | 1,560 | 2,300 | 1,380 | 0,680 | 0,950 |
| | Breite in mm | 0,018 | 0,013 | 0,015 | 0,013 | 0,010 | 0,011 |
| Glagah | Länge in mm | 2,300 | 1,310 | 1,850 | 1,260 | 0,820 | 1,040 |
| | Breite in mm | 0,026 | 0,018 | 0,020 | 0,018 | 0,013 | 0,015 |
| Kassoer | Länge in mm | 2,290 | 0,820 | 1,550 | 1,880 | 0,820 | 1,170 |
| | Breite in mm | 0,026 | 0,015 | 0,020 | 0,015 | 0,015 | 0,017 |

Die Tabellen II bis V zeigen, daß der Cellulosegehalt der Portion B viel höher ist als derjenige der anderen Portionen. Die Länge der Faser des äußeren

Teils des Zuckerrohrstengels ist bedeutend größer als die des inneren Teils. Die Portion A enthält die größte Menge Lignin; der Aschengehalt ist aber bei der Portion B geringer als bei den Portionen A, C und D. Aus diesen Versuchen können wir schließen, daß die Portion B das geeignetste Material für die Zellstoffherstellung bildet.

2. LEGUMINOSEPFANZEN.

Als Grundungen für das Zuckerrohr wurden im allgemeinen in Taiwan Leguminosepflanzen, wie *Crotalaria juncea*, *Crotalaria ussuriensis*, *Sesbania cannabina*, verwendet. Die Tabellen VIII und IX enthalten die Ergebnisse der Analyse dieser Pflanzen.

Tabelle VIII. *Crotalaria*.

| | <i>Crotalaria juncea</i> | | | <i>Crotalaria ussuriensis</i> | | |
|------------------------|--------------------------|------------|--------------|-------------------------------|------------|--------------|
| | Holzgewebe | Bastgewebe | Grüne Zweige | Holzgewebe | Bastgewebe | Grüne Gewebe |
| | In % der Trockensubstanz | | | | | |
| Stickstoff | 0,06 | 0,27 | 0,27 | 0,03 | 0,07 | 0,47 |
| Asche | 1,08 | 6,66 | 3,97 | 0,83 | 3,32 | 3,22 |
| Heißes Wasser-Extrakt | 14,31 | 30,07 | — | 13,19 | 29,84 | — |
| Verd. Alkali-Extrakt | 22,68 | 40,07 | — | 22,07 | 42,59 | — |
| Alkohol-Benzol-Extrakt | 5,15 | 9,81 | — | 4,01 | 9,48 | — |
| Pentosan | 17,50 | 11,53 | — | 19,69 | 12,76 | — |
| Lignin | 21,16 | 15,66 | — | 19,63 | 13,03 | — |
| Cellulose | 56,96 | 54,95 | — | 59,41 | 55,19 | — |

Tabelle IX. *Sesbania*.

| | <i>Sesbania cannabina</i> | | | <i>Sesbania sesban</i> |
|------------------------|---------------------------|------------|--------------|------------------------|
| | Holzgewebe | Bastgewebe | Grüne Zweige | |
| | In % der Trockensubstanz | | | |
| Stickstoff | 0,02 | 0,27 | 0,11 | 0,11 |
| Asche | 0,71 | 4,03 | 4,08 | 1,24 |
| Heißes Wasser-Extrakt | 12,51 | 35,45 | — | 14,01 |
| Verd. Alkali-Extrakt | 22,51 | — | — | 35,69 |
| Alkohol-Benzol-Extrakt | 4,81 | 10,81 | — | 4,50 |
| Pentosan | 19,41 | 13,69 | — | 19,44 |
| Lignin | 20,40 | 13,10 | — | 22,85 |
| Cellulose | 56,21 | 52,48 | — | 52,48 |

Pentosan- sowie Ligningehalt des Bastgewebes dieser Leguminosepflanzen sind geringer als diejenigen des Holzgewebes. Das Bastgewebe enthält aber größere Mengen von Stickstoff und Asche als das Holzgewebe.

Wir haben ferner die Veränderungen der chemischen Bestandteile im Laufe des Wachstums von *Crotalaria juncea* untersucht (Tabelle X).

Tabelle X.

| Tage nach dem Säen | 60 | | 90 | | 120 | |
|------------------------|--------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | Holz- gewebe | Bast- gewebe | Holz- gewebe | Bast- gewebe | Holz- gewebe | Bast- gewebe |
| | In % der Trockensubstanz | | | | | |
| Alkohol-Benzol-Extrakt | 1,88 | 4,41 | 2,87 | 6,38 | 2,65 | 7,80 |
| Heißes Wasser-Extrakt | 14,86 | 30,97 | 14,53 | 30,67 | 12,98 | 25,24 |
| Verd. Alkali-Extrakt | 34,60 | 53,04 | 32,74 | 50,58 | 25,65 | 46,22 |
| Lignin | 24,48 | 7,22 | 24,41 | 10,21 | 22,87 | 12,56 |
| Pentosan | 21,51 | 9,34 | 23,09 | 19,42 | 22,90 | 12,06 |
| Asche | 1,67 | 5,03 | 1,41 | 3,83 | 1,34 | 4,64 |
| Cellulose | 56,66 | 59,00 | 56,38 | 57,93 | 60,05 | 54,44 |
| In % der Cellulose | α -Cellulose | 70,94 | 84,40 | 74,87 | 89,55 | 78,55 |
| | β -Cellulose | Spur | Spur | Spur | Spur | Spur |
| | γ -Cellulose | 29,06 | 15,60 | 25,13 | 10,45 | 21,45 |
| Länge der Faser in mm | 0,81 | 4,90 | 0,89 | 4,96 | 0,93 | 5,04 |
| Breite der Faser in mm | 0,021 | 0,019 | 0,025 | 0,022 | 0,026 | 0,022 |

Aus diesen Ergebnissen geht hervor, daß für die Zellstoffherstellung das Bastgewebe geeigneter ist als das Holzgewebe, und daß das etwa 3 Monate alte Bastgewebe das geeignetste Material ist. Bemerkenswert ist die Tatsache, daß die Länge der Faser des Bastgewebes von *Crotalaria juncea* etwa 5 mm beträgt.

3. DIE ANDEREN PFLANZEN.

Es wurde Baumwollstaude, Rizinusstengel, Reisstroh, *Agave americana*, *Padanus odoratissimus* und *Casuarina stricta* analysiert (Tabelle XI).

Tabelle X.

| | Baum- woll- staude | Rizinus- stengel | Reisstroh | Agave americana | Padanus odoratis- simus | Casuarina stricta |
|------------------------|--------------------------|---------------------|-----------|--------------------|-------------------------------|----------------------|
| | In % der Trockensubstanz | | | | | |
| Asche | 2,80 | 4,72 | 16,54 | 15,25 | 5,25 | 1,25 |
| Heißes Wasser-Extrakt | 14,75 | 7,30 | 22,95 | 38,69 | 20,44 | 13,95 |
| Verd. Alkali-Extrakt | 28,45 | 33,46 | 54,26 | 60,69 | 43,34 | 28,45 |
| Alkohol-Benzol-Extrakt | 2,49 | 2,48 | 3,68 | 4,64 | 3,83 | 2,83 |
| Pentosan | 17,88 | 17,48 | 23,99 | 12,07 | 17,11 | 16,57 |
| Lignin | 26,80 | 22,84 | 17,11 | 12,52 | 19,98 | 26,10 |
| Cellulose | 56,71 | 53,48 | 43,24 | 36,15 | 44,54 | 56,09 |
| In % der Cellulose | α -Cellulose | 62,22 | 77,67 | — | — | 79,13 |
| | β -Cellulose | 8,05 | 5,93 | — | — | 7,80 |
| | γ -Cellulose | 29,73 | 16,49 | — | — | 13,07 |

ABSTRACTS

from

TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

Hydroxylation of Sorbic Acid. II.

Oxidation of Sorbic Acid by Pancreatic Acid.

(pp. 181~183)

By Masaru HAMADA.

(Osaka Factory, Sankyo Co., Ltd.: Received Feb. 9, 1940.)

Bromierung von Brenzschleimäure.

(SS. 184~186)

Von Yataro OBATA.

(Landwirtschaftliches Biochemisches Laboratorium der Kaiserl. Universität, Tokio,
Eingegangen am 9. 2. 1940.)

Zur Gewinnung von Tetrabromid der Brenzschleimsäure, wurde die Reaktion mit Brom unter verschiedenen Bedingungen versucht.

Die Behandlung mit Bromdämpfen wurde genau nach den Angaben von Tonnies,⁽¹⁾ Hill u. Sanger⁽²⁾ ausgeführt, doch gelang es uns nicht, im Gegensatz zu den Ergebnissen der genannten Verfasser, Tetrabromid zu erhalten.

Das Reaktionsprodukt war δ -Brombrenzschleimsäure (Fp. $184\sim 5^\circ$, Br 42.56% (41.88) Ausbeute 20%). Beim Arbeiten in eiskalter atherischer Lösung wurde auch ein δ -Bromsubstitutionsprodukt erhalten (Ausbeute 36.6%). Die Halogenierung anderer Verbindungen der Furan-Reihe wurde bei Tief-Temperatur ausgeführt. So wurde 24 g des vorher getrockneten Broms durch Kaltmischung bis zum Erstarren gekühlt und mit 6 g Brenzschleimsäure unter starkem Umrühren versetzt.

Der Inhalt wurde alsbald eine zane und alsdann eine gelbrote, voluminöse Masse. Überschüssiges unverwendbares Brom wurde durch Einleitung von getrockneter Luft befreit. Der Rückstand wurde mit Äther ausgezogen und mit gesättigter wässriger Lösung von Bisulfit sowie Wasser gewaschen. Der Ätherauszug wurde mit wasserfreiem Na_2SO_4 getrocknet und das Filtrat eingeeengt. Ließ man das Reaktionsprodukt unter Zusatz von Ligroin bei -10° auskrystallisieren, so wurden 22.5 g farbloser Krystalle vom Smp. $156\sim 158^\circ$ (Zers.) erhalten.

Es wurde durch Umkrystallisieren aus Benzol gereinigt, wobei Tetrabromid

vom Smp. $159.5 \sim 160^\circ$ (Zers.) erhalten wurde. Ausbeute 13.6 g (60%), Br 74.46%, 73.75% 73.75% (74.07).

Diese Versuche wurden von mir unter der Leitung von Herrn Prof. Bunsuke Suzuki ausgeführt, dem ich auch an dieser Stelle meinen besten Dank für seine Unterstützung aussprechen möchte.

SCHRIFTTUM.

- (1) Tonn es: B. **11**, 1086, (1878), **12**, 1207, (1879).
 - (2) Hill u. Sanger: B. **17**, 17591, (1884), A. **232**, 42, (1885).
 - (3) Paal: B. **17**, 2760, (1884)
- Saunders: J. Am. C. S. **15**, 133, (1898).

Hydroxylierungsversuche von Furanring.

(SS. 187~191)

Von Yataro OBATA.

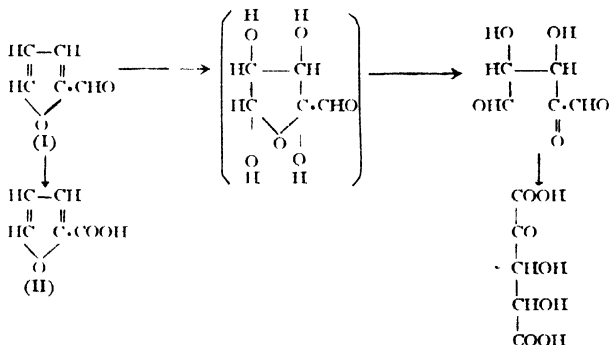
(Landwirtschaftl. Biochemisches Laboratorium der Kaiserl. Universität, Tokio;

Eingegangen am 9. 2. 1940.)

Von manchen Autoren⁽¹⁾ ist darauf hingewiesen worden, daß man von den Furanverbindungen zu den α -Ketonverbindungen unter Ringspaltung ohne Abbau der Kohlenstoff-Bindung kommt.

Wenn das Eintreten der Hydroxylgruppe an konjugierten Doppelbindungen von Furfural (I) sowie Brenzschleimsäure (II) gelingt, so wird die folgende Veränderung erwartet.

Dabei soll die stark reduzierende Substanz resultieren.



Es wurde zunächst so dargestellt, daß Furfural sowie Brenzschleimsäure mittels kalten alkalischen Permanganats in die Hydroxylverbindung übergeführt wurde. Dabei resultierte nicht das gewünschte Produkt, sondern es wurde Maleinsäure (Fp. $128 \sim 9^\circ\text{C}$, 41.62 (41.37) H 3.24 (3.44)) erhalten.

| Subst. (g) | Reaktionsdauer (Min.) | Ausbeute (g) |
|-------------------------------|-----------------------|--------------|
| Furfural 5 | | 2 |
| " 5 | 180 | 2 |
| Brenzschleimsäure 5 | 5 | 1.2 |
| Äthylester der obigen Säure 5 | 5 | 0.225 |

Die geringe Ausbeute aus Brenzschleimsäureäthylester ist auf die Schwerlöslichkeit in schwach alkalischer wässriger Lösung zurückzuführen. Die Darstellung des zu den Versuchen benutzten Tetrabromids von Brenzschleimsäure wurde bei (SS. 38 beschrieben,

Bei der Umsetzung des Tetrabromids mit feuchtem Silberoxyd wurde das stark reduzierende Produkt erhalten. Leider war die so entstandene Substanz ein nicht krystallisierbarer Sirup, der so labil war, daß er zu Oxalsäure abgespalten, gleichzeitig verharzt wurde.

Die Darstellung der krystallisierten Derivate war also nicht fruchtbar.

Das Reaktionsprodukt zeigt die Eigenschaften wie folgt:

- (1) ein schwach gelber Sirup von stark saurer Natur, der sich an der Luft zersetzt und mit der Erzeugung von Oxalsäure verharzt wird;
- (2) es wird rot bei alkalischer Reaktion;
- (3) es reduziert und entfärbt momentan das p-Dichlorphenolindophenol;
- (4) es reduziert Kaliumpermanganat, und Fehlingsche Lösung in der Kälte;
- (5) bei Jodtitration verbraucht die Probe, abgeleitet von 22.5 g Tetrabromid, 11.51 g Jod (= N/10 907 cc);
- (6) nach dem Bertrandschen Verfahren reduziert die Probe, abgeleitet von 22.5 g Tetrabromid, 3.991 g Cu;
- (7) die folgenden Reaktionen sind alle negativ mit diesem Produkt; Aldehyd, Zuckersäuredilakton⁽²⁾, Enolverbindung, Glykal, Glucosen.
- (8) Es erzeugt 2 M. Oxalsäure durch KMnO_4 -Oxydation⁽³⁾.

Nach der Entstehung von Glyoxylsäure durch Bleitetraacetat-Oxydation wurde auf das Vorkommen der COOH-CHOH-CHOH -Bindung geprüft.

Diese Untersuchungen wurden von mir unter der Leitung von Herrn Prof. Bunsuke Suzuki ausgeführt, dem ich hiermit für seine Unterstützung meinen besten Dank ausspreche.

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On the Soil Type of Mendoho in Northeast-Manchuria.

(pp. 192~196)

R. KAWASHIMA and G. SUYAMA.

(Agr. Chem. Laboratory, Kyushu Imp. University; Received Feb. 12, 1940.)

Mendoho is a village along the Harbin-Manchuria line and is situated in lat. $49^{\circ} 06'$ N. and long. $121^{\circ} 03'$ E. and is about 705 meters above sea-level. The climate is of the extreme continental type with hot summers and very cold winters. The annual rainfall varies considerably from a yearly mean of 44 8.7 mm. and mean annual temperature is -3.2°C .

Two soil types may be found, namely, a brown forest soil of both residual granitic and of alluvial deposit origin, and a steppe soil developed from loess-like material.

Of these, the brown forest soil of residual granitic is distributed most extensively.

The pH-values of these brown forest soils are nearly 7 and their lime status are good. Therefore, the agricultural value may be considered favourable.

In the steppe soil the zone of carbonate accumulation is found about 90 cm. below the surface.

On the Hydrolysis of Fats and Fatty Acid Esters. (VII)

(pp. 197~205)

By Toyoki ONO.

(Chemical Laboratory of the Fish Meal Association of Japan;

Received Feb. 26, 1940.)

The Relation between the Constitution of Glycerides and their Hydrolysis.

I. Distearin, monostearin, diolein and monoolein were obtained synthetically by Guth's method from dichlorohydrin, monochlorohydrin, potassium stearate and sodiumoleate. α -Oleodistearin was obtained from α -monoolein and stearic acid: β -oleodistearin and β -moroctodistearin from distearin and oleic acid or moroctic acid ($\text{C}_{18}\text{H}_{34}\text{O}_2$).

Tristearin, tripalmitin, triolein and dipalmitin were prepared by Berthelot's method as described in the previous paper.

II. The saponification velocity of triglycerides, diglycerides and monoglycerides of stearic, palmitic and oleic acids increases in the descending orders in the homogeneous and heterogenous system. The ratio of the reaction velocity coefficient of monostearin and tristearin is greater than that of monoolein and triolein.

III. Such mixed triglycerides as α -oleodistearin and β -moroctodistearin are split more rapidly than the simple triglycerides as tristearin. These facts seem to be due to the differences of the emulsification value or the affinity for alkali of glycerides. Table VI shows this explanation.

IV. The fatty acid radicals in α - and β -position in the β -morocotodistearin molecule are saponified with the same velocity in the homogenous system. In the heterogenous system, on the contrary, morocotic acid radical in β -position is split selectively more rapidly than the acid radical in α -position.

V. α -Oleodistearin and β -oleodistearin have no difference of the saponification velocity in homogenous system.

Table VI. The Hydrolysis of Glycerides and their Constitution.

| Time (min) | Tristearin | | Distearin | | Monostearin | | β -Morocotodistearin | |
|---------------|---------------|------------------|---------------|------------------|---------------|------------------|----------------------------|------------------|
| | by KOH (%) | by Lipase (%) | by KOH (%) | by Lipase (%) | by KOH (%) | by Lipase (%) | by KOH (%) | by Lipase (%) |
| 30 | 18.35 | — | 19.67 | — | 35.46 | — | 38.80 | — |
| 60 | 24.68 | 6.71 | 30.67 | 7.91 | 44.68 | 10.62 | 52.00 | 22.03 |
| 120 | 41.14 | 11.18 | 43.00 | 14.56 | 62.06 | 18.23 | 68.00 | 28.63 |
| 180 | — | 14.90 | — | 16.61 | — | 23.90 | — | 32.31 |
| 240 | 61.71 | — | 65.33 | — | 78.72 | — | 89.60 | — |
| 300 | — | 16.25 | — | 22.19 | — | 36.30 | — | 35.68 |

| Time (min) | Triolein | | Diolein | | Monoolein | | α -Oleodistearin | β -Oleodistearin |
|---------------|----------|--------|---------|--------|-----------|--------|-------------------------|------------------------|
| | KOH | Lipase | KOH | Lipase | KOH | Lipase | KOH | KOH |
| 30 | 25.07 | — | 25.57 | — | 28.67 | — | 32.00 | 34.78 |
| 60 | 39.20 | 44.31 | 44.59 | 46.78 | 50.67 | 50.00 | 45.00 | 46.39 |
| 120 | 58.64 | 50.22 | 59.67 | 57.52 | 65.00 | 60.00 | — | — |
| 180 | — | 54.65 | — | 65.95 | — | 68.92 | 64.00 | 60.87 |
| 240 | 81.79 | 60.56 | 82.62 | — | 82.67 | — | — | — |
| 300 | — | 60.56 | — | 72.09 | — | 75.68 | 70.00 | 72.46 |

The Discoloring Method for Melanin Containing Amino Acid Solution and its Application.

(pp. 206~208)

By K. KIHARA.

(Kagawaken Syoyu Laboratory; Received Feb. 3, 1940.)

I discovered that phenol is an excellent absorptive solvent for melanin.

Add baryta water to the melanin containing amino acid solution, and filter off the precipitated BaPO_4 . After shaking the filtrate with 1/3 of its volume of phenol in a separating funnel vigorously, stand for a few minutes, then the contents separate in two layers, the upper phenol and the lower aqueous solution. To this aqueous solution, ether is added, shaken vigorously to extract the mixing phenol, and then concentrated on water bath. Take 5 cc from this concentrated solution, dilute to 50 cc with water, and determine the amino nitrogen by the micro Van Slyke method. (Reaction with HNO_2 for 3 minutes, absorption in KMnO_4 -KOH solution for 2 minutes each by vigorously shaking).

Calculate the amino N in 100 cc concentrated solution; This is *B*.

To 20 cc of the concentrated solution, add 2 gr baryta and dissolve by warming, and then add 80 cc 94% alcohol. The precipitate is filtered off by means of suction pump, washed with 80% alcohol, dried at 40°C in drying oven. The dried mass is dissolved in acetic aqueous solution by warming, dilute to 500 cc, determine amino N in 2 cc, calculate the glutamic acid N in 100 cc concentrated solution. This is *A*. Dilute 5 cc of the original melanin containing amino acid solution to 50 cc, determine the amino N in 2 cc, calculate the amino N in 100 cc original solution. This is *C*.

A, *B*, and *C* are mgr numbers at 760 mm 16°C. The glutamic acid N of the original melanin containing amino acid solution is $A/B \times C$ mg. This is taste number. A/B : taste ratio.

| For example | taste number |
|---|--------------|
| Japanese soya | 183.6 |
| HCl hydrolysis products of soy bean protein | 518.7 |
| HCl hydrolysis products of wheat prote n | 611.6 |

On the Denaturation of Sericin. (Part 1)

Study of the denaturation of sericin caused by boiling in hot water.

(pp. 209~212)

By ZIRÔ HIROSE.

(The Sericultural Research Laboratory of Gunze Raw Silk Mfg. Co., Ltd;

Received Feb. 26, 1940.)

1. Introduction.

When the raw cocoon layers were divided into 3 parts of equal weight, namely, the outside, the middle and the inside layer, we found by experiments that the solubility in water of the sericin retained in the outside layer of the cocoon was always greater than that of either of the other 2 layers. This is, according to the views of F. Haurowitz,⁽¹⁾ mainly due to the difference in ionic structures and in hydrophylic groups among the 3 layers. Keeping in mind that the absorption power of sericin for the tanning agent depends mainly upon the physico-chemical properties of its hydrophylic groups, we studied the absorption power of the sericin retained in the outside and the inside layers of the raw cocoon by treating with basic chromium sulphate (Cationic chromate complex).

Experimental results were as follows;

5 g of the outside and the inside layers of the cocoon were treated with 200 cc of the 33.77% basic chromium sulphate solution, kept at 20°C for 16 hours.

| Concentration (Cr_2O_3 g/L) of chrome solution | Cr_2O_3 absorbed per 100 g of sericin retained on the outside layer (g) | Ditto to the sericin retained on the inside layer (g) |
|--|---|--|
| 1.53 | 4.58 | 4.05 |
| 3.06 | 7.67 | 6.22 |
| 5.59 | 9.52 | 8.40 |

The remarkable difference of the absorption power of the sericin between the outside and the inside layers of the raw cocoon was obtained, confirming the theory of F. Haurowitz⁽¹⁾.

Haurowitz also stated that the denaturation of native proteins caused by boiling in hot water was due to the modification of the ionic structures and the hydrophylic groups. So we can easily imagine, when the sericin denatured by boiling in hot water was treated with some tanning agents, its absorption power for those agents may be different from the original one corresponding to the degree of the denaturation.

2. Study of denaturation by boiling in hot water of the sericin retained in the raw cocoon layer.

A. Pretreatment with boiling water (Process of denaturation.)

Raw cocoon layers were treated with boiling water for 15 minutes and 30 minutes respectively. Then those pretreated cocoon layer were centrifuged and cooled. As soon as cooled at room temperature, those cocoon layers were treated with the chrome solutions.

B. Chromination.

We studied the denaturation, caused by treating with boiling water, of the sericin retained in the raw cocoon layers by studying the absorption power of those sericins for basic chromium sulphate (cationic chromate complex) and basic oxalato-chromiate⁽²⁾ (anionic chromiate complex) respectively.

1. In the case of the basic chromium sulphate.

5g of the cocoon layers were treated with 200 cc of the 33.77% basic chromium sulphate solution of 1.530 g/l. Cr_2O_3 concentration, kept at 20°C for 3 hours.

| Time of pretreatment with boiling water | O. | 15 minutes | 30 minutes |
|--|------|------------|------------|
| Cr_2O_3 absorbed per 100 g of the sericin retained on the cocoon layer (g) | 3.63 | 3.23 | 2.92 |

2. In the case of the basic trans-oxalato-chromiate.

5g of the cocoon layers were treated with 200 cc of 28.74% basic trans-oxalato chromiate of 1.139 g/l. Cr_2O_3 concentration, kept at 20°C for 3 hours.

| Time of pretreatment with boiling water | O. | 15 minutes | 30 minutes |
|--|------|------------|------------|
| Cr_2O_3 absorbed per 100 g of the sericin retained on the cocoon layer (g) | 2.14 | 2.81 | 3.18 |

SUMMARY.

The work included in this paper may properly be summed up as follows.

1. The sericin retained in the outside layer of the raw cocoon takes up more cationic chromate complex than that retained in the inside layer of the raw cocoon.

2. Sericin retained in the cocoon layers, denatured by treating with boiling water, takes up more anionic chromate complex and minor cationic chromate complex than the original one, corresponding to the degree of denaturation.

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Amylo-Process in Out-Door Sealed Tank.

(pp. 213~223)

By Yosito TAKEDA and Iwao NAITO.

(Monopoly Bureau, Government General of Taiwan; Received Feb. 27, 1940.)

Researches on Foliaceous Woods as Raw Material for Pulp in Taiwan. (Part I)

(pp. 224~226)

By Minoru TUTIVA and Yoshiteru KATO.

(Industrial Research Institute of Taitzu, Taiwan, Japan; Received Feb. 26, 1940.)

On the Carbohydrate in Lobster- and Crab-meat-protein.

(pp. 227~232)

By Kinsuk KONDO and Uichiro SARATA.

(Nutritional Chemical Laboratory, Faculty of Agriculture and Chemical Institute,
Kyoto Imperial University; Received Feb. 3, 1940.)

Studies on the Absorption Spectra of Ovovitellin of Hen and Quail.

(pp. 233~234)

By Kinsuke KONDO, Sakae SHINANO, and Hisateru MITSUTA.

(Nutritional Chemical Laboratory, Faculty of Agriculture and Chemical Institute,
Kyoto Imperial University; Received Feb. 3, 1940.)

The Effect of Glutathione upon Alcoholism.
(Biochemical Studies on Glutathione. The XIIth Report.)
(pp. 238~244)

By Masayoshi OGAWA.

(Department of Nutrition, College of Medicine, Nippon University;
Received Feb. 22, 1940.)

In the present communication the author describes an experiment on the effect of glutathione upon alcoholism, employing a number of male albino rats weighing about 100 grams, which were intoxicated by subcutaneous injections of alcohol (C_2H_5OH) (0.1~0.5 cc. per 100 grams of the body weight), obtaining the following results.

Rate of Intoxication.

| Body weight, (grams) | Alcohol injected (cc) | (per 100 grams of the body weight) | GSH injected (mg) | (per 100 grams of the body weight) | Times observed, (hours) | | | | | | | | | |
|-------------------------|-----------------------------|--|-------------------------|--|-------------------------|-----------|---------|-------------|---------|-------------|---------|-------------|---------|-------------|
| | | | | | 0~ 1/2 | 1/2~ 1 | 1~1.1/2 | 1.1/2~ 2 | 2~2.1/2 | 2.1/2~ 3 | 3~3.1/2 | 3.1/2~ 4 | 4~4.1/2 | 4.1/2~ 5 |
| 88 | 0.1 | 0.1 | 0 | 0 | + | + | + | ± | | | | | | |
| 84 | 0.1 | 0.1 | 12.5 | 15.0 | + | + | + | ± | | | | | | |
| 73 | 0.15 | 0.2 | 0 | 0 | + | + | + | ± | | | | | | |
| 74 | 0.15 | 0.2 | 10.1 | 15.0 | + | + | + | ± | | | | | | |
| 75 | 0.25 | 0.3 | 0 | 0 | ± | ± | ± | + | + | | | | | |
| 75 | 0.25 | 0.3 | 10.3 | 15.0 | ± | ± | ± | ± | + | + | | | | |
| 81 | 0.33 | 0.4 | 0 | 0 | + | ± | ± | ± | + | + | | | | |
| 83 | 0.33 | 0.4 | 12.5 | 15.0 | ± | ± | ± | ± | ± | ± | ± | ± | + | |
| 98 | 0.50 | 0.5 | 0 | 0 | + | ± | ± | ± | ± | ± | ± | ± | + | |
| 95 | 0.50 | 0.5 | 14.2 | 15.0 | ± | ± | ± | ± | ± | ± | ± | ± | ± | |
| 75 | 0.38 | 0.5 | 0 | 0 | + | + | ± | ± | ± | ± | ± | ± | + | |
| 100 | 0.50 | 0.5 | 0 | 0 | + | ± | ± | ± | ± | ± | ± | ± | + | |
| 95 | 0.48 | 0.5 | 0 | 0 | + | ± | ± | ± | ± | ± | ± | ± | + | |
| 103 | 0.50 | 0.5 | 0.5 | 0.5 | + | ± | ± | ± | ± | ± | ± | ± | ± | + |
| 94 | 0.48 | 0.5 | 0.47 | 0.5 | ± | ± | ± | ± | ± | ± | ± | ± | ± | + |
| 85 | 0.43 | 0.5 | 0.85 | 1.0 | ± | ± | ± | ± | ± | ± | ± | ± | ± | + |
| 110 | 0.55 | 0.5 | 1.10 | 1.0 | ± | ± | + | ± | ± | ± | ± | ± | ± | + |
| 85 | 0.43 | 0.5 | 4.30 | 5.0 | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± |
| 100 | 0.50 | 0.5 | 5.00 | 5.0 | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± |
| 98 | 0.50 | 0.5 | 10.0 | 10.0 | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± |
| 100 | 0.50 | 0.5 | 20.0 | 20.0 | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± |
| 97 | 0.50 | 0.5 | 30.0 | 30.0 | ± | ± | ± | ± | ± | ± | ± | ± | ± | ± |

As shown in the above table, the animals injected with alcohol and GSH, became more deeply intoxicated than those injected with alcohol only.

By injecting a large dose such as 0.6 or 0.7 cc. of alcohol per 100 grams of

the body weight, and at the same time injecting GSH in doses of 0 mg., 5 mg., 10 mg., 20 mg., respectively per 100 grams of the body weight, the author obtained the following results :

Death or Recovery of the Animals.

| Body weight | Alcohol injected (cc) $\left(\begin{smallmatrix} \text{per 100} \\ \text{grams of} \\ \text{the body} \\ \text{weight} \end{smallmatrix}\right)$ | | GSH injected (mg) $\left(\begin{smallmatrix} \text{per 100} \\ \text{grams of} \\ \text{the body} \\ \text{weight} \end{smallmatrix}\right)$ | | Recovery | Death | Death Rate (%) |
|-------------|--|-----|--|----|----------|-------|----------------|
| 98 | 0.58 | 0.6 | 9.8 | 10 | — | + | 40 |
| 96 | 0.58 | 0.6 | 9.6 | 10 | + | — | |
| 94 | 0.55 | 0.6 | 9.4 | 10 | + | — | |
| 103 | 0.63 | 0.6 | 10.3 | 10 | — | + | |
| 97 | 0.58 | 0.6 | 9.7 | 10 | + | — | |
| 126 | 0.75 | 0.6 | 0 | 0 | + | — | 0 |
| 90 | 0.55 | 0.6 | 0 | 0 | + | — | |
| 107 | 0.65 | 0.6 | 0 | 0 | + | — | |
| 120 | 0.73 | 0.6 | 0 | 0 | + | — | |
| 105 | 0.63 | 0.6 | 0 | 0 | + | — | |
| 96 | 0.67 | 0.7 | 0 | 0 | — | + | 80 |
| 110 | 0.77 | 0.7 | 0 | 0 | — | + | |
| 125 | 0.88 | 0.7 | 0 | 0 | + | — | |
| 90 | 0.63 | 0.7 | 0 | 0 | — | + | |
| 124 | 0.88 | 0.7 | 0 | 0 | — | + | |
| 115 | 0.81 | 0.7 | 0 | 0 | + | — | |
| 81 | 0.57 | 0.7 | 0 | 0 | — | + | |
| 95 | 0.67 | 0.7 | 0 | 0 | — | + | |
| 107 | 0.75 | 0.7 | 0 | 0 | — | + | |
| 131 | 0.90 | 0.7 | 0 | 0 | — | + | |
| 79 | 0.55 | 0.7 | 7.9 | 10 | — | + | 100 |
| 69 | 0.48 | 0.7 | 6.9 | 10 | — | + | |
| 79 | 0.55 | 0.7 | 7.9+7.9 | 20 | — | + | |
| 77 | 0.53 | 0.7 | 7.7+7.7 | 20 | — | + | |
| 95 | 0.70 | 0.7 | 4.8 | 5 | — | + | |
| 83 | 0.60 | 0.7 | 8.3 | 10 | — | + | |
| 81 | 0.60 | 0.7 | 16.2 | 20 | — | + | |

As shown in the above table, the animals injected with alcohol (0.7 cc per 100 grams of the body weight) and GSH, became extremely intoxicated, and all of them died (death rate was 100%), but of the control animals injected with alcohol only, 80% died.

Chemical Studies on Clay-soil under Water. (Part 1)

Manurial Effects of Clay-soil under Seawater.

(pp. 245~248)

By Masayoshi ISHIBASHI.

(Chemical Institute, College of Science, Kyoto Imperial University ;

Received Feb. 9, 1940.)

Formation of β -Hydroxy-pyridine Derivatives from Hexoses and NH_3 -Salts.

[II] 2-Hydroxymethyl-5-hydroxy-pyridine.

(pp. 249~252)

[III] 2-Methyl-5, 6-dihydroxy-pyridine.

(pp. 253~264)

By Kiyosi Aso.

(Agricultural Chemical Laboratory, Tokyo Imperial University ; Received Feb. 22, 1940.)

Berichtigungen.

Band 16, Heft 2.

Die Synthese von α - und β -Glycerophosphorsäure.

Von Yataro ORATA.

S. 30, Z. 4, v. u. „Eingegangen“ statt „Eigegangen.“

„ Z. 4, v. o. (ausschließlich der Formeln).

„gezeigt, gestaltete das α -Ba-Salz nicht das schwerlösliche Doppelsalz, aber“
statt „gezeigt gestaltete das α -Ba-Salz nicht das schwerlösliche Doppelsalz aber“

S. 31, Z. 11, v. o. (ausschließlich der Formeln).

„Im Gegensatz bedurfte die Oxydation mit Perjodsäure nur weniger Minuten (15 Min.) und ist deshalb viel nützlicher. Die Substanz ist das α -Ba-Salz, hergestellt vom kaulfichen Ca-Salz (Merck) nach der Methode von Karrer und Salomon.⁽¹⁾“ statt „Im Gegensatz bedurfte die Oxydation mit Perjodsäure nur weniger Minuten (15 Min.) und α -Ba-Salz, ist deshalb der Methode von Karrer und Salomon.⁽²⁾“

S. 32, Z. 6, v. o. „Schütteln“ statt „Schuttenln.“

„ Z. 23, v. o. „30 cc“ statt „30 cm.“

„ „ „ „50 cc“ statt „50 cm.“

„ Z. 25, „ „ β -Ba-Salz“ statt „ β -Ba-Salz.“

„ Z. 42, „ „Giorn. Chim. Ind. Appl.“ statt
„Giorn. Chem. Ind. Appl.“

„ Z. 2, v. u. „Während der Korrektur“ statt

„Während die Korrektur.“

Bulletin of the Agricultural Chemical Society of Japan.

TRANSACTIONS

Über die Bestandteile des Zuckerrohrs.

VON K. HONDA, T. TATSUNO, Y. NAKAMURA, T. GODA,
Y. MIMA und K. YAMAFUJI.

(Aus dem Institut für Zuckerforschung in Tainan.)

Eingegangen am 9. 3. 1940.

Das Zuckerrohr kann neben der Zuckerproduktion auch für die Herstellung von Alkohol, Zellstoff, Furfural, Glycerin, Aceton usw. benutzt werden. Eingehende Angaben über die physikalischen Eigenschaften sowie die chemischen Bestandteile des Zuckerrohrstengels sind aber sehr spärlich. In der vorliegenden Arbeit haben wir einige im hiesigen Institut gezüchtete vorzügliche Varietäten von *Saccharum officinarum* als Versuchsmaterialien gewählt und zunächst morphologische sowie chemische Untersuchungen ausgeführt. Dann wurde die Herstellung des Zellstoffs aus der Bagasse versucht.

1. MORPHOLOGISCHE UNTERSUCHUNGEN DES ZUCKERROHRSTENGELS.

Das 18 Monate alte Zuckerrohr wurde gepreßt, die erhaltene Bagasse mit Schulz'scher Lösung behandelt und die Form der Bagassezelle mikroskopisch beobachtet. Auf Grund dieser Beobachtung haben wir die Bagassezellen in drei Gruppen geteilt und die Zahl der zu jeder Gruppe gehörigen Zellen bestimmt (Tabelle I).

Tabelle I.

| Varietät des Zuckerrohrs | F 108 | F 109 | F 110 | F 111 | Mittel |
|--------------------------|-------|-------|-------|-------|--------|
| Faserzelle in % | 42,90 | 54,08 | 47,79 | 35,05 | 44,66 |
| Rechteckige Zelle in % | 29,31 | 18,96 | 24,45 | 34,17 | 27,02 |
| Weiche Zelle in % | 27,39 | 26,96 | 27,76 | 30,78 | 28,32 |

Ferner wurden die durchschnittliche Länge und Breite dieser Zellen ermittelt (Tabelle II).

Tabelle II.

| | | F 108 | F 109 | F 110 | F 111 |
|-------------------|--------------|-------|-------|-------|-------|
| Faserzelle | Länge in mm | 1.172 | 1.051 | 1.138 | 1.155 |
| | Breite in mm | 0.018 | 0.018 | 0.018 | 0.018 |
| Rechteckige Zelle | Länge in mm | 0.305 | 0.287 | 0.325 | 0.343 |
| | Breite in mm | 0.053 | 0.066 | 0.070 | 0.068 |
| Weiche Zelle | Länge in mm | 0.231 | 0.205 | 0.221 | 0.268 |
| | Breite in mm | 0.147 | 0.133 | 0.130 | 0.140 |

Die Resultate der genaueren Bestimmungen enthalten die Tabellen III, IV und V.

Tabelle III.

Länge der Faser.

| Länge in mm | F 108 | F 109 | F 110 | F 111 |
|-------------|--------------------------|-------|-------|-------|
| | In % der gesamten Zellen | | | |
| 0, — 0.25 | 0 | 0 | 0 | 0 |
| 0.25 — 0.50 | 6.87 | 6.30 | 9.31 | 6.91 |
| 0.50 — 0.75 | 24.89 | 23.40 | 23.08 | 18.43 |
| 0.75 — 1.00 | 19.31 | 24.47 | 19.84 | 26.27 |
| 1.00 — 1.25 | 10.73 | 19.50 | 9.72 | 13.82 |
| 1.25 — 1.50 | 13.73 | 8.87 | 11.34 | 12.90 |
| 1.50 — 1.75 | 9.01 | 8.16 | 8.91 | 7.37 |
| 1.75 — 2.00 | 5.15 | 4.96 | 8.50 | 6.45 |
| 2.00 — 2.25 | 2.58 | 2.13 | 3.64 | 3.23 |
| 2.25 — 2.50 | 3.43 | 1.42 | 4.80 | 0.46 |
| 2.50 — 2.70 | 1.29 | 1.06 | 0 | 1.84 |
| 2.75 — 3.00 | 2.58 | 0 | 0 | 0.46 |
| 3.00 — 3.25 | 0 | 0 | 0.41 | 1.38 |
| 3.25 — 3.50 | 0.43 | 0 | 0 | 0 |
| 3.50 — 3.75 | 0 | 0 | 0.41 | 0.46 |

Tabelle IV.

Breite der Faser.

| Breite in mm | F 108 | F 109 | F 110 | F 111 |
|-----------------|--------------------------|-------|-------|-------|
| | In % der gesamten Zellen | | | |
| 0, — 0.01 | 6.8 | 6.5 | 6.5 | 6.0 |
| 0.01 — 0.0125 | 22.2 | 10.5 | 14.5 | 15.5 |
| 0.0125 — 0.0150 | 13.0 | 12.0 | 13.5 | 14.5 |
| 0.0150 — 0.0175 | 14.0 | 12.5 | 17.0 | 12.5 |
| 0.0175 — 0.0200 | 9.2 | 19.5 | 15.5 | 16.5 |
| 0.0200 — 0.0225 | 18.4 | 18.0 | 13.5 | 15.5 |
| 0.0225 — 0.0250 | 4.8 | 7.0 | 8.0 | 8.0 |

| | | | | |
|-----------------|-----|-----|-----|-----|
| 0,0250 — 0,0275 | 2,9 | 3,0 | 3,0 | 2,0 |
| 0,0275 — 0,0300 | 1,5 | 3,0 | 1,5 | 3,0 |
| 0,0300 — 0,0325 | 3,4 | 4,0 | 4,5 | 3,0 |
| 0,0325 — 0,0350 | 1,5 | 1,0 | 1,0 | 0,5 |
| 0,0350 — 0,0375 | 0 | 0,5 | 1,0 | 0,5 |
| 0,0375 — 0,0400 | 0,5 | 1,0 | 0,5 | 0 |
| 0,0400 — 0,0425 | 0 | 1,0 | 0 | 2,5 |
| 0,0425 — 0,0450 | 1,5 | 1,0 | 0 | 0 |
| 0,0450 — 0,0475 | 0,5 | 0,5 | 0 | 0 |

Tabelle V.
Länge der rechteckigen Zelle.

| Länge in mm | F 108 | F 109 | F 110 | F 111 |
|--------------------------|-------|-------|-------|-------|
| In % der gesamten Zellen | | | | |
| 0 — 0,1 | 0,43 | 0,64 | 0,30 | 0,25 |
| 0,1 — 0,2 | 21,74 | 18,59 | 11,62 | 11,00 |
| 0,2 — 0,3 | 40,00 | 47,50 | 39,38 | 36,33 |
| 0,3 — 0,4 | 23,91 | 22,44 | 31,61 | 29,90 |
| 0,4 — 0,5 | 9,13 | 5,77 | 10,36 | 14,47 |
| 0,5 — 0,6 | 1,74 | 1,92 | 3,63 | 5,47 |
| 0,6 — 0,7 | 1,74 | 1,20 | 1,55 | 5,47 |
| 0,7 — 0,8 | 0,87 | 0,80 | 1,80 | 1,93 |
| 0,8 — 0,9 | 0,40 | 0,50 | 0,24 | 0,64 |
| 0,9 — 1,0 | 0,03 | 0,64 | 0,32 | 0 |

2. CHEMISCHE UNTERSUCHUNGEN DES ZUCKERROHRSTENGELS.

Zunächst wurden das spezifische Gewicht und der Zuckergehalt des aus dem 18 Monate alten Zuckerrohr gewonnenen Saftes ermittelt (Tabelle VI).

Tabelle VI.

| | F 108 | F 109 | F 110 | F 111 |
|------------|-------|-------|-------|-------|
| Grade Brix | 21,9 | 19,7 | 19,8 | 21,3 |
| Grade Pol | 20,1 | 18,9 | 18,8 | 20,2 |

Die Bagasse wurde nach der vollständigen Entfernung des Zuckers auf übliche Weise analysiert (Tabelle VII u. VIII).

Tabelle VII.

| | F 108 | F 109 | F 110 | F 111 |
|--------------------------|-------|-------|-------|-------|
| In % der Trockensubstanz | | | | |
| Asche | 1,49 | 1,39 | 1,55 | 1,64 |
| Kalt-Wasser-Extrakt | 2,54 | 1,94 | 2,32 | 2,24 |

Tabelle X.

| Dauer der max. Temp. in Std. | 1 | 2 | 3 | 5 | | |
|---------------------------------|---------------------|-------|-------|-------|-------|-------|
| Ausbeute in % | 36,52 | 35,14 | 33,36 | 30,97 | 29,12 | |
| In % des Zellstoffs | | | | | | |
| Pentosan | 12,72 | 11,53 | 11,45 | 11,05 | 11,02 | |
| Lignin | 3,82 | 3,09 | 2,92 | 2,70 | 2,02 | |
| Asche | 2,11 | 1,41 | 1,42 | 1,55 | 1,17 | |
| Cellulose | 92,57 | 92,77 | 93,53 | 95,91 | 97,41 | |
| In % der Cellulose | α -Cellulose | 85,30 | 82,75 | 78,75 | 74,78 | 76,41 |
| | β -Cellulose | 11,44 | 13,96 | 16,05 | 22,60 | 20,22 |
| | γ -Cellulose | 3,27 | 3,29 | 5,20 | 2,62 | 3,37 |

Der Soda- und Zuckergehalt der nach dem Aufschluß erhaltenen Lösung sind in Tabelle XI verzeichnet.

Tabelle XI.

| Dauer der max. Temp. in Std. | 1/2 | 1 | 2 | 3 | 5 |
|--------------------------------------|------|------|------|------|------|
| NaOH in % | 22.2 | 20.0 | 17.9 | 15.2 | 12.4 |
| Na ₂ CO ₃ in % | 3.2 | 4.0 | 4.2 | 6.4 | 7.2 |
| Reduzierender Zucker in % | 1.84 | 1.18 | 1.00 | 1.59 | 2.16 |
| Gesamtzucker in % | 4.78 | 3.92 | 4.05 | 4.37 | 4.60 |

Wir haben dann die Bagasse vor dem Aufschluß mit heißem Wasser vorbehandelt. Zu diesem Zwecke wurden 100 g Bagasse mit 500 cm Wasser gekocht. Die chemische Zusammensetzung der so vorbehandelten Bagasse ist die folgende (Tabelle XII).

Tabelle XII.

| Max. Druck in Pfunden | 30 | | | 60 | | |
|----------------------------------|---------------------|-------|-------|-------|-------|-------|
| Dauer des max. Drucks in Std. | 2 | 3 | 4 | 1 | 2 | |
| Ausbeute in % | 78,31 | 76,93 | 74,11 | 74,46 | 70,46 | |
| In % der Trockensubstanz | | | | | | |
| Verd. Alkali-Extrakt | 31,27 | 30,85 | 30,48 | 31,87 | 31,63 | |
| Lignin | 21,91 | 21,28 | 20,83 | 21,75 | 21,74 | |
| Pentosan | 14,46 | 15,07 | 12,83 | 11,67 | 11,79 | |
| Asche | 3,38 | 2,25 | 1,69 | 2,35 | 1,59 | |
| Cellulose | 48,16 | 46,71 | 45,34 | 44,66 | 42,28 | |
| In % der Cellulose | α -Cellulose | 81,84 | 82,19 | 82,85 | 85,04 | 88,20 |
| | β -Cellulose | 16,94 | 16,41 | 15,25 | 13,77 | 10,26 |
| | γ -Cellulose | 1,22 | 1,41 | 1,90 | 1,19 | 1,54 |

Die Ergebnisse der Aufschlußversuche mit dieser vorbehandelten Bagasse sind in Tabelle XIII zusammengefaßt.

Tabelle XIII.

| Max. Druck in Atm. | Dauer des max. Drucks 2 Stunden | | | | | | |
|-----------------------|---------------------------------|------------------|---------------------|--------|-------|-----------|---------------------|
| | NaOH in % | Ausbeute in % | Pentosan | Lignin | Asche | Cellulose | α -Cellulose |
| | | | In % des Zellstoffs | | | | |
| 5 | 6 | 43,3 | 7,80 | 2,40 | 1,89 | 92,09 | 90,10 |
| | 4 | 45,5 | 11,29 | 3,17 | 1,77 | 94,35 | 92,04 |
| | 3 | 48,7 | 13,43 | 3,84 | 1,69 | 93,51 | 91,74 |
| | 2 | 51,5 | 14,55 | 5,61 | 1,89 | 90,17 | 89,14 |
| 6 | 6 | 42,1 | 7,64 | 1,72 | 1,12 | 93,65 | 86,94 |
| | 4 | 44,3 | 10,99 | 2,21 | 1,09 | 93,66 | 88,62 |
| | 3 | 47,2 | 12,07 | 2,51 | 1,34 | 92,46 | 88,96 |
| | 2 | 51,6 | 14,05 | 4,48 | 1,08 | 91,30 | 87,74 |
| 6,5 | 6 | 40,2 | 7,70 | 1,68 | 1,28 | 91,87 | 86,01 |
| | 4 | 41,4 | 9,91 | 2,19 | 1,79 | 93,63 | 88,05 |
| | 3 | 44,5 | 10,34 | 2,28 | 1,92 | 94,83 | 88,93 |
| | 2 | 47,3 | 10,42 | 3,25 | 1,90 | 92,04 | 88,99 |

Die Bleichungsversuche wurden in folgender Weise durchgeführt: Der Zellstoff wurde mit dem gleichen Gewicht von Wasser gemischt und eine Stunde bei Zimmertemperatur mit Chlorgas behandelt. Hierauf wurde derselbe mit verdünnter Natriumhydroxydlösung gekocht und dann wieder mit Bleichpulver gebleicht. Der gebleichte Zellstoff wurde auch analysiert (Tabelle XIV).

Tabelle XIV.

| Aufschlußbedingungen | | Ausbeute nach der Bleichung in % | α -Cellulose | β -Cellulose | γ -Cellulose | Pentosan | Asche |
|-----------------------|--------------|---|---------------------|--------------------|---------------------|----------|-------|
| Max. Druck in Atm. | NaOH in % | | In % des Zellstoffs | | | | |
| 6,5 | 6 | 94,7 | 88,75 | 5,41 | 5,84 | 9,96 | 0,88 |
| | 4 | 94,9 | 89,10 | 6,21 | 4,69 | 13,24 | 0,76 |
| | 3 | 93,2 | 87,50 | 7,29 | 5,21 | 13,94 | 1,04 |
| | 2 | 90,8 | 82,37 | 9,13 | 8,50 | 15,19 | 1,15 |
| 6 | 6 | 95,2 | 89,69 | 5,30 | 5,01 | 9,06 | 1,08 |
| | 4 | 95,4 | 89,63 | 5,71 | 4,66 | 10,28 | 1,01 |
| | 3 | 92,6 | 86,03 | 6,20 | 7,77 | 11,51 | 1,13 |
| | 2 | 90,8 | 82,70 | 8,70 | 8,60 | 14,75 | 1,09 |
| 5 | 6 | 96,8 | 90,51 | 4,21 | 5,28 | 7,98 | 0,98 |
| | 4 | 96,8 | 91,69 | 4,63 | 3,68 | 9,87 | 0,86 |
| | 3 | 92,5 | 91,05 | 4,71 | 4,24 | 11,01 | 0,85 |
| | 2 | 90,7 | 89,10 | 5,70 | 5,20 | 12,41 | 1,01 |

Influence of Monochromatic Light on the Action of Enzymes.

Especially Influence of Monochromatic Light on the Action of Yeast Enzymes.

By Reitaro MURAKAMI.

(Chemical Laboratory, Agricultural College, Utunomiya.)

Received March 6, 1940.

In order to study the effects of spectral monochromatic light on the action of yeast enzymes, saccharase, proteinase, catalase, amylase, and lipase were extracted by autolyzing "Oriental" pressed yeast, the first three of which were refined as follows. In the case of saccharase, after the liquefaction was completed by the addition of 10% of toluene to 225 g of the yeast, the same volume of water was added and the whole allowed to autolyze at room temperature for 7 days. The precipitate obtained by adding an equal volume of alcohol to the autolyzed liquid was extracted with 20% alcohol. Alcohol was then added to the extract, and the saccharase extracted from the second precipitate with 100 cc of water.

In the case of proteinase, after 250 g of the yeast was liquefied by the addition of 10% of ethyl acetate, 500 cc of water was added and the acid that had formed in 2 hours was neutralized continuously by the addition of ammonia water and then a solution that rendered ineffective the tryptic and ereptic actions was separated out and washed with water. The yeast was then suspended in water containing toluene and allowed to autolyze at room temperature for 24 hours. An acetic solution was added to the autolyzate obtained by filtration, and after the pH of the liquid was made 5.0, a *N*/15 acetate buffer of pH 5.0 and aluminium hydroxide were added. The proteinase was finally obtained by elution of the adsorbate with 44 cc of secondary ammonium phosphate.

Catalase was prepared by liquefying yeast. By adding to 150 g of it 33% of toluene the yeast was liquefied within 1 hour at 40°C, after which 200 cc of water was added, and the whole allowed to autolyze overnight in a refrigerator. To the autolyzate was added 44 cc of *N*/10 hydrochloric acid, and the total volume of liquid made up to 2000 cc with water to which aluminium hydroxide was finally added. The catalase was obtained by elution of the adsorbate with 400 cc of *M*/30 phosphate solution (pH=7.6).

Amylase and lipase were prepared by autolyzing for 1 day at room temperature and then overnight in a refrigerator after adding 10% of toluene and 2.5 times the volume of water to 200 g of the yeast.

As substrate for the saccharase, 20% saccharose dissolved in 1% primary sodium phosphate solution was used. Into a test tube containing 4 cc of the substrate, 1 cc of the enzyme solution was added, and the pH of the resulting solution

adjusted to 4.24. The substrate for the amylase was 1% soluble starch solution. Into a test tube containing 10 cc each of the substrate and phosphate buffer solution, 10 cc of the enzyme solution was added, and the pH of the solution adjusted to 6.71. The substrate for the proteinase was 5% gelatine solution. Into a test tube containing 5 cc each of the substrate and citrate buffer, 1 cc of the enzyme solution was added, the resulting pH of the solution being 5.1. The substrate for the lipase, which was castor oil or olive oil, was neutralized with *N*/20 sodium hydroxide. Into a test tube containing 5 cc of the substrate, 5 cc of the enzyme solution was added, and the pH of the resulting solution adjusted to 7.4. The substrate for catalase was *N*/20 hydrogen peroxide buffered with phosphate mixture, the pH of which was 6.7. One cc of the enzyme solution was added to a test tube containing 20 cc of the substrate. The test tubes were placed within tin boxes, and filters placed at the front windows of the boxes. The boxes containing the test tubes were incubated at from 20° to 40°C, according to the particular enzyme, the door opened, and then lighted by a lamp through filters and a 2 cm layer of *N*/10 copper sulphate solution (except in the case of work on infra-red rays), as the copper solution absorbs infra-red rays from a distance of 1 meter.

As the light source, a nitra lamp (Mazda C 100~300 watt and O. K. 500 watt) was used for visible rays. "Vim Ray" blue and red lamps (each 300 watt) used respectively for ultra-violet and infra-red rays.

The filters were made by spreading 7 cc of gelatine solutions, containing various pigments per 1 square dm, over colourless glass or "Acme ultra vit glass" plates which were used respectively for the purpose of visible and infra-red rays or ultra-violet rays, and then drying by means of a fan. The amounts of pigment per 70 cc of gelatine solution are shown in Table I.

Table I. Composition of the filters.

| Plate | Filter | Pigment |
|------------------------------|---------------------------|---|
| Glass plate | White filter | Aesculin 0.2 g or gelatine alone |
| | Infra-red pass filter | Filter blue 0.1 + filter yellow 0.1 + toluidine blue 0.01 g |
| | Red filter | Rhodamine 0.42 + tartrazine 0.42 + erythrosine 0.42 g |
| | Green filter | Patent blue 0.2 + tartrazine 0.7 g |
| | Blue filter | Patent blue 0.2 + rhodamine 0.7 + aesculin 0.2 g |
| | Violet filter | Methyl violet 0.42 + toluidine blue 0.17 + aesculin 0.2 g |
| | Black filter | India ink 3.5 cc |
| "Acme ultra vit glass" plate | Ultra-violet close filter | Aesculin 0.2 g |
| | Ultra-violet pass filter | Nitrosodimethylaniline 0.03 + toluidine blue 0.06 + copper sulphate 0.874 g |

As to the wave lengths of the transmission rays passed through these filters, they were spectroscopically examined; the photograms, which were taken by means of a constant deviation wave length spectrometer and a quartz spectrograph, are

Needless to say, if enzyme enter into special substrate, of which a portion of the molecules kept the state of the stable E naturally, it can be changed to the active state E' at a certain temperature without radiant energy, and then changes a portion of S by the action of E' to the labile state S' . The change of E to E' increases as a temperature rise to optimum; consequently it increases a possibility to change S to S' .

In case the enzyme received radiant energy possessing suitable vibration, E' is increased, and it changes S plentifully to S' . Thus, decomposition of substrate advances more by the action of the enzyme receiving suitable radiant energy than by the opposite and it represents promotive effect.

If, however, the enzyme happens to meet with the radiant energy possessing a too great and unsuitable vibration, E changes to E'' , and the enzyme is destroyed. Thus, the action of enzyme on substrate becomes weaker than that which has not received radiant energy and it represents an inhibiting effect.

It is shown in such relation as

$$E''_{(min)} > E' > E$$

$E''_{(min)}$ is the state reached the acting limit, as seen in the case of radiant energy in the near ultra-violet region on saccharase, amylase and catalase. The region of wave length to reach E'' varies with the kinds of enzymes. It is seen that E' is plentifully formed, owing to the radiant energy in the near ultra-violet on proteinase and lipase, in spite of E not changing to E'' except in the region of shorter wave length.

The increase of the promotive effect under the same coloured visible lights with increase in the relative intensities of the absorbed rays, depends upon E' being abundantly formed along with the relative intensities, and also the effect per unit intensity of various coloured visible lights is due to E' being formed approximately proportional to wave number of the absorbed rays.

In conclusion, the writer wishes to express his thanks to Prof. U. SUZUKI for his interest and encouragement, and also to the Imperial Academy for grants given in aid of this study.

ABSTRACTS

from

TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

Über Vitamin C im Tee.

(SS. 265~270)

Von Akiji FUJITA und Isamu NUMATA.

(Biochemisches Laboratorium des Kitasato Institutes, Tokyo, Eingegangen am 19. 3. 1940)

On the Carbohydrate in Hen's Ovovitellin.

(pp. 271~276)

By Kinsuke KONDO and Uichiro SARATA.

(Nutritional Chemical Laboratory, Faculty of Agriculture and Chemical Institute, Kyoto Imperial University; Received Mar. 6, 1940.)

Die Abhängigkeit der Enzymentwicklung, insbesondere von Amylase und Protease, von der Art der Gerste und ihrer Keimung.

(SS. 277~280)

Von H. NAKAMURA.

(Aus dem Laboratorium der Dainippon Brauerei; Eingegangen am 27. Feb. 1940.)

1) Die Entwicklung von Amylase und Protease bei der Keimung ist abhängig von der Art der Gerste, wodurch auch das Verhältnis der bezuglichen Enzymkräfte untereinander sehr verschieden sein kann.

| Beispiel: | Wurzellänge bezogen auf Kornlänge: | Protease: | Amylase: |
|-----------|--|-----------|----------|
| Gerste A: | 1,6 | 5,3 | 3,2 |
| Gerste B: | 1,6 | 2,3 | 7,7 |

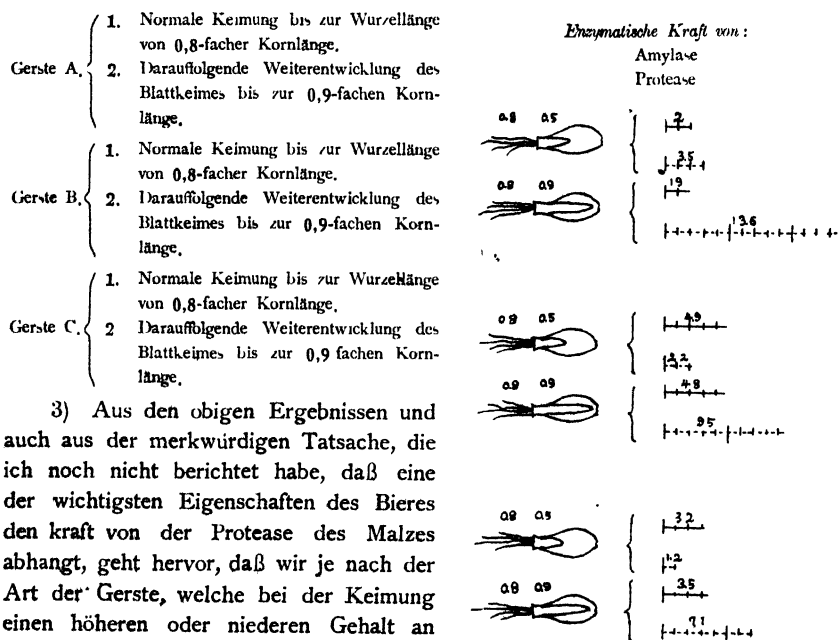
Wenn bei der Gerste B die Amylasenwirkung zur Proteasewirkung mit 1:1 gesetzt wird, so ergibt sich bei der Gerste A die Relation der gleichen Wirkung mit 1:5,5.

2) Wenn der Wurzelkeim nach Erreichung einer bestimmten Länge im Wach-

sen behindert wird, der Blattkeim sich aber weiterentwickelt, dann hört die Bildung der Amylase ganz auf, dagegen nimmt die Protease in einem erstaunlichen Umfang zu.

Falls beispielsweise der Wurzelkeim einer Gerstenart bei der Keimung nur bis zur 0,8-fachen Kornlänge wächst und der Blattkeim daraufhin bis zur 0,9-fachen Kornlänge vorgetrieben wird, so tritt in der Bildung von Amylase ein Stillstand ein, wogegen die Protease eine weitere Zunahme—und zwar um das 4-bis 6-fache—erfährt. Dementsprechend ist dann auch die enzymatische Kraft des nach diesem Verfahren erreichten Proteasegehaltes eine vielfach größere als die beim Höchstgehalt an Protease, welche bei der natürlich gekeimten Gerste entsteht.

Bei Anwendung dieses Verfahrens auf verschiedene Gerstenarten unterliegen die absoluten und relativen Werte von Amylase und Protease weitgehenden Schwankungen.



3) Aus den obigen Ergebnissen und auch aus der merkwürdigen Tatsache, die ich noch nicht berichtet habe, daß eine der wichtigsten Eigenschaften des Bieres den kraft von der Protease des Malzes abhängt, geht hervor, daß wir je nach der Art der Gerste, welche bei der Keimung einen höheren oder niederen Gehalt an Protease entwickelt, bestrebt sein müssen, diese Bildung an Protease genau zu überwachen und zu lenken. Die Erreichung dieses Zieles setzt die Ausarbeitung entsprechender neuer Kontrollverfahren voraus.

On Alcoholic Fermentation of Acorn by Amylo Process.

(pp. 281~287)

By Seisaku SUGIZAKI.

(Agricultural Chemical Laboratory, Department of Agriculture, Tokyo Imperial University;

Received March 6, 1940)

Studies on the Vegetable Tannins in Taiwan. Part 5.

Manufacture of Tanning Extract from the Bark of *Acacia confusa*. I.

(pp. 288~292)

By Yasuyosi OSIMA, Minoru ISII and Zenyu Hyo.

(Agricultural Chemical Department, Taihoku Imperial University, Taiwan,

Received March, 19, 1940)

We found that the maximum yield of tannin was obtained by extracting the dried bark with 50% alcohol. From fresh bark extraction with water gives equally good yield but the extraction was far easier and very much faster with 50% alcohol.

The maximum temperatures for extraction of the materials are as follows:

| | |
|---------------------------------|----------|
| Dried bark extracted with water | 80°C. |
| " " 50% alcohol | 60~80°C. |
| Fresh bark " " | 80°C. |

On a New Polypeptide Isolated from *Eisenia Bicyclis*.

(Part III)

A Study of the Chemical Structure of Eisenin. (2)

(pp. 293~298)

By Toshihiko OOHIRA.

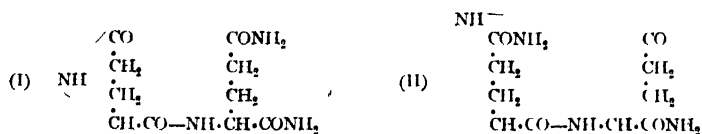
(Agricultural Chemical Laboratory, Tokyo Imperial University,

Received Mar. 12, 1940.)

For the determination of the amino acid having a free carboxyl group in eisenin molecule, eisenin was converted into thiohydantoin derivative by means of ammonium thiocyanate and acetic acid anhydride; then it was decomposed by 25% aqueous ammonia solution, according to the method by P. Schlack and W. Kumpf. The ammonia was removed from the solution by evaporation under reduced pressure, and from the residue two different crystals A [m.p. 160~161°] and B [m.p. 240~241° (decomp.)] were obtained; the "A" from the ether extract and the "B" from the residue. The "A" was identified as 5-methyl-2-thiohydration by com-

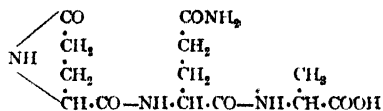
paring with an authentic specimen prepared from alanine. The "B" was found to be a new dipeptide $C_{10}H_{16}O_4N_4$ having two acid-amide groups.

The formation of 5-methyl-2-thiohydantoin from eisenin shows that alanine is in the end position of eisenin molecule; its amino group only being substituted for the peptide grouping. Therefore it is suggested that the chemical structure of the substance, obtained by partial hydrolysis with 3% aqueous bariumhydroxide solution as has been mentioned in the previous paper, may be di- $[\alpha$ -amino- γ -carboxy-butyl]-alanine. It is presumed also that the dipeptide $C_{10}H_{16}O_4N_4$, mentioned above, may correspond to one of the two structures shown below,



To settle this question, the author prepared *l*-pyroglutamyl-*d*-glutamic acid diamide representing the structure (I), *l*-pyroglutamic acid chloride (1 mol) obtained by the action of thionylchloride on *l*-pyroglutamic acid, was treated with *d*-glutamic acid diethylester (2 mols) in chloroform solution. The reaction mixture was successively washed with dilute hydrochloric acid and aqueous sodium bicarbonate solution and then the solvent was distilled off. The residual crystalline mass was dissolved in a little alcohol and mixture of ether and petroleum ether added to it. The crude crystals of *l*-pyroglutamyl-*d*-glutamic acid diethylester separated out, were recrystallized from acetic ester. This diethylester was treated with 25% aqueous ammonia solution at room temperature for about two hours. After the solution was evaporated to a small volume at about 30~40° under diminished pressure, absolute alcohol was added to the residue until a crystal was separated out by stirring. It was recrystallized from dilute alcohol. This crystal was proved to be completely identical with the substance obtained from thiohydantoin derivative of eisenin by the treatment with ammonia.

From these results, *l*-pyroglutamyl-*d*-glutamyl-*d*-alanine may be given as the chemical structure of eisenin.



Sterilizing Action of Phenols.

Synopsis.

(pp.299 ~305)

By Sogo TETSUMO¹⁰.

(Government Institute for Infect Dis., Tokyo Imper. University, Received Feb. 27, 1940)

I reported previously concerning the sterilizing action of mineral acids and fatty acids. Then I studied the sterilizing action of phenol group as noted in the following table.

I. REAGENTS

TABLE 1. Phenols

| Number of OH | Phenols | Rational formulae | M W | weight % at N/1000 |
|--------------|-------------------------|---|---------|--------------------|
| 1 | Phenol | C_6H_5OH | 94.048 | 0.0094 |
| | Cresol (o) | $C_6H_4 \begin{matrix} OH \\ \\ CH_3 \end{matrix}$ (1) | 108.064 | 0.0108 |
| | Guaiacol | $C_6H_3 \begin{matrix} OH \\ \\ OCH_3 \end{matrix}$ (1) | 124.064 | 0.0124 |
| | Thymol | $C_6H_3 \begin{matrix} OH \\ \\ CH(CH_3)_2 \end{matrix}$ (1) | 162.167 | 0.0162 |
| | Picric acid | $C_6H_2(NO_2)_3 \cdot OH$ (2) | 215.044 | 0.0215 |
| 2 | Pyrocatechin (o) | $C_6H_2 \begin{matrix} OH \\ \\ OH \end{matrix}$ (1) | 110.048 | 104° |
| | Resorcin (m) | $C_6H_4 \begin{matrix} OH \\ \\ OH \end{matrix}$ (2) | " | 110° |
| | Hydroquinone (p) | $C_6H_4 \begin{matrix} OH \\ \\ OH \end{matrix}$ (3) | " | 170° |
| | Pyrogalllic acid (o) | $C_6H_3 \begin{matrix} OH \\ \\ OH \\ \\ OH \end{matrix}$ (4) | 126.048 | 132° |
| 3 | Phloroglucine (m) | $C_6H_3 \begin{matrix} OH \\ \\ OH \\ \\ OH \end{matrix}$ (1) | " | 218° |
| | Hydroxyhydroquinone (p) | $C_6H_4 \begin{matrix} OH \\ \\ OH \\ \\ OH \end{matrix}$ (2) | | |
| | | Hydroxyhydroquinone, was not available and the experiment was not performed | | |

II. STERILIZING ACTION OF PHENOLS AT THE SAME CONCENTRATION

The sterilizing power and special character of phenols at the same concentration, were studied in this experiment, and the results obtained are shown in the following table.

Picric acid, cresol and thymol are insoluble at N/100, so they were used in this experiment at N/1000. Only phenol was tested at N/10 and N/100.

TABLE 2. Sterilizing Action of Phenols at the Same Concentration.
N/1000. (Phenol. N/10, N/100) 20°C.

| * Number of OH | Phenols | Conc | pH | Surviving period | | | |
|----------------|----------------------|--------|------|-------------------------------------|-------------------------------------|-------------------------------------|-------------------------------------|
| | | | | <i>Staph. pyogen.</i> | <i>Prot. vulgar.</i> | <i>Bac. typhos.</i> | <i>Vib. cholera</i> |
| 1 | Phenol | N/10 | 5.17 | 5 ^m + 10 ^m - | 1 ^m + 2 5 ^m - | 2.5 ^m ± 5 ^m - | 1 ^m - |
| | " | N/100 | 5.21 | 4 ^d + 5 ^d - | 2 ^d + 3 ^d - | 4 ^d ± 5 ^d - | 90 ^m + 2 ^h - |
| | " | N/1000 | 5.45 | 7 ^d ± 8 ^d - | 4 ^d + 5 ^d - | 5 + 6 - | 9 ^h ± 12 ^h - |
| | Cresol (o) | " | 5.36 | 6 + 7 - | 4 ± 5 - | 5 + 6 - | 6 ± 9 - |
| | Guaiacol | " | 6.13 | 10 + 12 - | 7 + 8 - | 8 + 10 - | 24 ± 36 - |
| | Thymol | " | " | 2 ^h + 3 ^h - | 60 ^m ± 90 ^m - | 90 ^m + 2 ^h - | 1 ^m ± 2 5 ^m - |
| | Picric acid | " | 2.30 | 90 ^m + 2 ^h - | 30 + 45 - | 60 ± 90 ^m - | 1 ± 2 5 ^m - |
| 2 | Pyrocatechin (o) | " | 5.31 | 18 ^h + 24 ^h - | 9 ^h ± 12 ^h - | 12 ^h + 18 ^h - | 10 ^m ± 15 ^m - |
| | Resorcine (m) | " | 5.57 | 13 ^d + 15 ^d - | 8 ^d + 12 ^h - | 10 ^d + 12 ^d - | 24 ^h ± 15 ^m - |
| | Hydroquinone (p) | " | 5.64 | 12 ^h + 18 ^h - | 6 ^h + 9 ^d - | 9 ^h + 12 ^h - | 15 ^m ± 20 ^m - |
| 3 | Pyrogalllic acid (o) | " | 4.58 | 2 ^d + 3 ^d - | 18 ^h + 28 ^h - | 1 ^d + 2 ^l - | 30 ± 45 ^m - |
| | Phloroglucine (m) | " | 5.71 | 20 ~ 25 | 10 ^d ~ 15 ^d | 15 ~ 20 | 24 ^h + 36 ^h - |
| Control | | | | 8 ^d ± | 5 ^d ± | 6 ^d ± | 18 ^h ± |

From the results noted in table 2 we know the following facts:—

The sterilizing action of phenol, which is usually used as the most popular disinfectant in hygiene, is strong only at N/10, but at lower concentrations the sterilizing action diminishes very distinctly, and at N/1000 concentration phenol has no sterilizing power on bacteria except for *Vib. cholera*.

The order of the strength of the sterilizing action at the same concentration of phenols is as follows:

Picric acid > Thymol > Hydroquinone > Pyrocatechin > Pyrogalllic acid. Cresol, guaiacol, resorcine and phloroglucine have totally no bactericidal action at N/1000. Especially resorcine and phloroglucine have a remarkable promoting action on the life of the microorganisms.

Relation between the chemical constitution and the strength of the sterilizing action is as follows. From the results of 2(OH)phenols and 3(OH) phenols, we see the following order as to the sterilizing power.

Para > Ortho > Meta.

III. STERILIZING ACTION OF PHENOL SALTS AND PHENOL ANIONS.

The action of phenol salts and of phenol anions on the life of bacteria were tested in this experiment. Concentration of Na, Ca and NH₄ salts, having the same anions as phenols was made N/1000.

TABLE 3. I. Na salts.

| Na | Surviving period | | | |
|-----------------|-----------------------------------|-----------------------------------|-----------------------------------|-------------------------------------|
| | <i>Staph pyogen</i> | <i>Prot vulgar</i> | <i>Bar typhus</i> | <i>Vib cholera</i> |
| phenolate | 7 ^d - 9 ^l | 5 ^l - 6 ^l | 6 ^l - 7 ^l | 9 ^h + 12 ^h - |
| o-cresolate | 7 - 9 | 5 - 6 | 6 - 7 | 9 + 12 - |
| guaiacolate | 13 - 15 | 7 - 9 | 10 - 12 | 24 + 36 - |
| thymolate | 6 ^h ± 9 ^h - | 2 ^h + 3 ^h - | 3 ^h + 6 ^h - | 5 ^m ± 10 ^m - |
| picrate | 13 ^l - 15 ^l | 8 ^d - 10 ^d | 12 ^l - 15 ^l | 24 ^h + 36 ^h - |
| pyrocatechnate | 10 - 12 | 8 - 10 | 10 - 13 | 12 + 18 - |
| resorcinate | 15 - 18 | 10 - 12 | 12 - 15 | 24 + 36 - |
| hydroquinonate | 5 + 6 - | 2 + 3 | 4 + 5 - | 2 + 3 - |
| pyrogallate | 10 - 12 | 6 - 8 | 8 - 10 | 12 + 18 - |
| phloroglucinate | 30 - 35 | 20 - 25 | 25 - 30 | 36 + 48 - |
| | 8 ^l ± | 5 ^d ± | 6 ^l ± | 18 ^h ± |

The results with Ca salts and NH₄ salts are nearly the same as with Na salts and they need not be recorded here

IV STERILIZING ACTION OF PHENOLS AT THE SAME pH

The sterilizing action of phenols was tested at the same pH in this experiment. In reference to the sterilizing action at a high pH, I record the results with thymol

Results obtained shown in the following table

TABLE 4. Sterilizing action of phenols at the same pH.

| Phenols | Conc | pH | Surviving period | | | |
|-----------------|----------|------|-------------------------------------|-------------------------------------|------------------------------------|-------------------------------------|
| | | | <i>Staph pyogen</i> | <i>Prot vulgar</i> | <i>Bar typhus</i> | <i>Vib cholera</i> |
| Phenol | N/1000 | 5.45 | 7 ^d + 8 ^l - | 4 ^l + 5 ^d - | 5 ^l + 6 ^d | 9 ^h ± 12 ^h - |
| Picric acid | N/200000 | " | 4 + 5 - | 3 ± 4 - | 3 + 4 - | 3 + 6 - |
| o-Cresol | N/2000 | " | 8 + 9 - | 5 ^d + 6 ^d - | 6 + 7 - | 9 + 12 - |
| Pyrocatechin | N/4000 | 5.64 | 2 + 3 - | 24 ^h + 36 ^h - | 2 ^l ± 3 ^d - | 30 ^m + 45 ^m - |
| Resorcin | N/1500 | " | 14 + 15 - | 9 ^l ± 10 ^d - | 12 ± 13 - | 24 ^h + 36 ^h - |
| Hydroquinone | N/1000 | " | 12 ^h + 18 ^h - | 6 ^h + 9 ^h - | 9 ^h ± 12 ^h - | 2 ^{5m} ± 5 ^m - |
| Pyrogallic acid | N/150000 | 5.71 | 15 ^d - 18 ^d | 7 ^d - 10 ^d | 12 ^d - 15 ^d | 12 ^h + 18 ^h - |
| Phloroglucine | N/1000 | " | 20 - 25 | 10 - 15 | 15 - 20 | 24 + 36 - |
| Thymol | N/1000 | 6.13 | 2 ^h + 3 ^h - | 60 ^m ± 90 ^m | 90 ^m + 2 ^h - | 1 ^m ± 2 ^{5m} - |
| Control | | | 8 ^d | 5 ^d ± | 6 ^d | 18 ^h ± |

V. SUMMARY.

The results noted in tables 2~3, and 4, concerning the sterilizing action of phenols and their salts, may be summarised as follows:

(1). At the same concentration, the sterilizing action of picric acid is the strongest among 10 phenols, and next to this is thymol. The sterilizing action of hydroquinone is slightly weaker than thymol, and pyrocatechin and then pyrogallol acid come next to thymol.

(2). Sterilizing action of phenol is strong only at $N/10$, and at concentration lower than $N/100$ it has no sterilizing power.

(3). The sterilizing action of guaiacol and resorcinol is very feeble and rather seems to have no power except for *Vib. cholerae*. At $N/1000$ they have rather evidently promoting power for bacterial life.

Phloroglucinol has totally no bactericidal action, but has rather evidently promoting action on bacterial life.

(4). Salts of hydroquinone have relatively strong sterilizing action, which is more evident with salts of thymol.

Salts of phenols other than thymol and hydroquinone, have no bactericidal action, and show rather promoting action on the bacterial life.

From these facts we see that anions of thymol and hydroquinone have bactericidal action.

(5). The strong sterilizing action of picric acid is chiefly due to the low pH of picric acid in addition to the poisoning action of molecular state of picric acid.

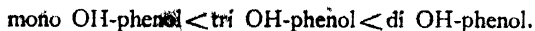
The strong sterilizing action of thymol is chiefly due to the poisoning action of molecular state of thymol and partly due to the action of thymol anion.

(6). The relative strength of the sterilizing action of *o*, *m*, and *p* isomers such as pyrocatechol (*o*) and resorcinol (*m*), and hydroquinone (*p*), and also pyrogallol acid (*o*) and phloroglucinol (*m*), are as follows:

$$m < o < p.$$

The cause of these differences is due to the difference of chemical constitution of each isomer.

(7). The relation between the strength of the sterilizing action of phenols and the number of OH group which give the acid character to phenols may be expressed as follows:



Biochemical Investigation of Mosaic Disease of Tobacco Plants. VI.

On Ascorbic Acid Oxydase and Saccharase in the Leaves of Healthy and Mosaic Plants.

(pp.306 ~310)

By Y. OKUDA, K. KATAI and E. MURATA.

(Agricultural Chemical Laboratory, Kyushu Imperial University, Received March 11, 1940.)

Über die Verwitterung der Eruptivgesteine. VI

Über den Verwitterungskomplex.

(SS. 311~320)

Von Mituru HARADA.

(Landwirtschaftliche Hochschule Tottori; Eingegangen am 22. 3. 1940.)

Der $\text{SiO}_2\text{-Al}_2\text{O}_3$ -Niederschlag wurde wie folgt hergestellt. 1 N. AlCl_3 -Lösung wurde mit Na-Silikatlösung bekannten Gehalten titriert, bis sich der Niederschlag abgesetzt und die darüber stehende Flüssigkeit klar geworden ist. Zwecks Gewinnung von Niederschlägen mit niedrigem Verhältnis $\text{SiO}_2\text{:Al}_2\text{O}_3$ wurde eine Lösung gebraucht, die Na-Silikat und NaOH enthält. Der Niederschlag wurde filtriert und mit 95%igem Alkohol Cl-frei gelassen, alsdann der Alkohol durch Einblasen von Luft verjagt. Der $\text{SiO}_2\text{-Fe}_2\text{O}_3$ -Niederschlag wurde in gleicher Weise hergestellt.

Beim $\text{SiO}_2\text{-Al}_2\text{O}_3$ -Niederschlag ist die Löslichkeit der Kieselsäure in der Oxalsäure-Kaliumoxalatlösung (18.4 g $\text{K}_2\text{C}_2\text{O}_4\cdot\text{H}_2\text{O}$, 3.2 g $\text{H}_2\text{C}_2\text{O}_4\cdot 2\text{H}_2\text{O}$ im Liter) um so größer, je kleiner das Mol.-Verhältnis $\text{SiO}_2\text{:Al}_2\text{O}_3$ ist. Niederschläge, deren Mol.-Verhältnis kleiner als 2 ist, sind fast ganz löslich, dagegen ist beim Niederschlag mit dem Verhältnis 6 nur 11% der Kieselsäure löslich. Die Tonerde wurde zu 87% beim Mol.-Verhältnis 6, zu 99~100% beim Verhältnis 0,8~2,3 durch die Oxalsäure-Kaliumoxalatlösung gelöst. Beim $\text{SiO}_2\text{-Fe}_2\text{O}_3$ -Niederschlag ist das Eisen fast ganz, die Kieselsäure aber nur 28~58% in der Oxalsäure-Kaliumoxalatlösung löslich.

Durch wiederholtes Trocknen und Anfeuchten wurde die Kieselsäure im $\text{SiO}_2\text{-Al}_2\text{O}_3$ -Niederschlag mit einem Mol.-Verhältnis $\text{SiO}_2\text{:Al}_2\text{O}_3$ größer als 2 schwer löslich, dagegen wurde diese Löslichkeit bei Mol.-Verhältnis kleiner als 2 nicht verändert; die Löslichkeit der Tonerde wurde bei Mol.-Verhältnis größer als 6 vermindert. Durch dieselbe Behandlung im Dunkeln wurde die Löslichkeit der Kieselsäure im $\text{SiO}_2\text{-Fe}_2\text{O}_3$ -Niederschlag allgemein stark Verminderung der Löslichkeit des Eisens sehr gering. In dem in Oxalsäure Kaliumoxalatlösung löslichen Teil ist das $\text{SiO}_2\text{/Al}_2\text{O}_3$ Verhältnis kleiner als 2 (0,6~2,0) und das $\text{SiO}_2\text{/Fe}_2\text{O}_3$ Verhältnis ist etwa 1 (0,6~1,1). Während das nicht getrocknete frische Aluminium-

hydroxyd in der Oxalsäure-Kaliumoxalatlösung löslich ist, wird es durch wiederholtes Austrocknen und Befeuchten unlöslich. Eisenhydroxyd lost sich in dieser Oxalatlösung im Dunkeln auf, und die Veränderung der Löslichkeit nach wiederholtem Trocknen und Befeuchten ist sehr gering.

Aus den obigen Ergebnissen wird erhellt, daß die Tonerde im $\text{SiO}_2\text{-Al}_2\text{O}_3$ -Niederschlag und Eisen im $\text{SiO}_2\text{-Fe}_2\text{O}_3$ -Niederschlag nur schwache Alterungsvorgänge zeigen und wie das frisch gefällte Hydroxyd chemisch reagiert, während die Kieselsäure schnell altert.

Der in Boden gebildete Verwitterungskomplex besteht aus 3 Fraktionen, nämlich aus einem Komplex (A_1), löslich in der Oxalsäure Kaliumoxalatlösung im Dunkeln, einem Komplex (A_2), zersetzbar in heißer konzentrierter Salzsäure, aber unlöslich in dieser Oxalatlösung, und einem Komplex (B), nur zersetzbar in heißer konzentrierter Schwefelsäure.

Der Komplex A_1 im Boden wird folgendermaßen bestimmt. Man wägt 0,5~1 g des zerkleinerten Bodens in einer Stohmanschen Halbliterflasche, übergießt den Boden mit 250 cm der Oxalsäure-Kaliumoxalatlösung, schüttelt im Dunkeln 1/2 Stunde lang aus und filtriert, alsdann bestimmt man SiO_2 und Al_2O_3 in der Lösung. Das gelöste Fe_2O_3 ist fast ganz im freien Zustande.

Freies Eisenoxyd (E) (s. Mitteilung IV) und die freie Tonerde (T), die in heißer 10%iger Na_2CO_3 -Lösung löslich ist, werden bestimmt.

Komplex A = (das in konz. Salzsäure zersetzbare SiO_2 , Al_2O_3
und Fe_2O_3) - A_1 - E - T

SiO_2 , Al_2O_3 und Fe_2O_3 im Komplex A_1 , A_2 und B in verschiedenen Bodenarten auf Eruptivgesteinen wurden bestimmt. Vulkanische Aschenboden enthalten Komplex A_1 , A_2 und B. Die Boden aus Hornblende-Andesit sind reich an Komplex A_1 und A_2 , Granit-, Diorit-, Quarztrachyt-, Augit-Andesit- und Basalt-Boden enthalten Komplex A_1 und B. Die Boden aus lockerem vulkanischen Lapilli enthalten große Mengen von Komplex A_1 . Quarzkeratophyrboden ist reich an Komplex A_2 .

Der Verf. hat gefunden, daß der Humus im Boden in Gegenwart von Eisenhydroxyd durch 3%ige H_2O_2 -Lösung oxydiert wird, wobei ein Teil des Aluminium in Komplex A_1 als Oxalat in Lösung geht, und ferner durch die folgende Behandlung das ganze Aluminium in Komplex A_1 aber kein gealtertes Aluminiumhydroxyd gelöst wird. 0,5~1 g Boden, 0,5 g Hydrochinon, 0,05 g Eisenhydroxyd und 60 cm 3%ige H_2O_2 -Lösung wird in ein Becherglas gebracht, mit einem Uhrglase bedeckt und auf siedendem Wasserbade erhitzt. Beim Steigen der Temperatur bis zu 70~80° reagiert das Gemisch energisch. Ist die Zersetzung des H_2O_2 beendet, wird nach Zusatz von 1 g NH_4Cl filtriert und das gelöste Aluminium bestimmt.

Studies on Acetone-Butylalcohol Fermentation. (III).

Utilization of various protein-rich raw materials as
N-source for acetone-butylalcohol fermentation.

(pp. 321~330)

By Sigeyosi HORIE.

(Agricultural Chemical Laboratory, Kyusyu Imperial University, Fukuoka ;

Received March 25, 1940.)

On the Colorimetric Determination of Vitamin B₁.

(pp. 331~339)

By Yosito SAKURAI, Tyoten INAGAKI and Sizu OMORI.

(Research Laboratory of Meiji Sugar Co ; Received March 22, 1940)

The procedure described by Prebluda and Melnick which involves the use of diazotized *p* aminoacetophenone as the reagent for the determination of vitamin B₁ is modified to the following simpler method, concentrating the vitamin B₁ in the extract by the adsorption on acid clay.

The sample is extracted with water or dilute alcohol at pH 4.5. An aliquot portion of the extract is adsorbed with 0.2 g refined acid clay for about ten minutes, and then centrifuged. To the centrifuged adsorbate 3 cc of water, 3 cc of alcohol containing phenol and 6 cc of freshly prepared reagent are added. The reaction is complete in 1 hour, after which 8 cc of alcohol and 5 cc of xylene are added followed by vigorous stirring for 2 minutes. On standing the xylene layer separates out easily showing pink color. After clarification by centrifugation the xylene layer is taken in 1 cm cuvette and the extinction is measured by Pulfrich's photometer using S 53 filter (pale green).

The extinction coefficient of 30 micrograms of vitamin B₁ hydrochloride is as follows.

| | Without acid clay | With 0.2 g acid clay |
|---------|------------------------------------|------------------------------------|
| Average | 0.323 (average of 16 measurements) | 0.280 (average of 12 measurements) |
| Maximum | 0.337 | 0.299 |
| Minimum | 0.302 | 0.267 |

**Über die Synthese von α -Naphthylensäure, β -Indolyl-
buttersäure und β -Indolylpropionsäure.**

(SS. 340~344)

Von Kinjiro TAMARI.

(Landwirtschaftliches Chemisches Laboratorium der Kaiserl. Universität, Tokyo,

Eingegangen am 26. 3. 1940)

Bulletin of the Agricultural Chemical Society of Japan.

ABSTRACTS

from

TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noted.)

On the Retting of Vegetable Fibre Materials.

The Useful Bacteria for the Retting of Kenaf.

(pp. 345~348)

By Tosio NAKAHAMA.

(Kanebo Yamashina Institute; Received Apr. 1, 1940.)

Twelve strains of aerobes and ten strains of anaerobes were isolated from the fermenting vat.

In carrying out pure fermentations of kenaf with these bacteria, one strain of aerobic bacillus and one strain of anaerobic coccus were selected as the most useful organisms.

According to their morphological, cultural and physiological characteristics, these bacteria were found to be new species, and named *Listerella hibiscus liquefaciens* and *Micrococcus hibiscus* respectively.

Studies on Sucrose Diet. (II)

(pp. 349~368)

By Yosito SAKURAI and Sizu OMORI.

(Research Laboratory of Meiji Sugar Co.; Received March 22, 1940.)

The rats fed on the following synthetic diet containing exhausted cane molasses as the source of vitamin B complex except B₁ and B₂, grew normally, indicating that the diet is complete in all respects.

| | |
|---------------|----|
| Sucrose | 65 |
| Fish protein | 15 |
| Butter fat | 10 |
| Salts mixture | 5 |
| Cane molasses | 5 |

Daily supplements

| | |
|--------------------------------------|---|
| Vitamin B ₁ hydrochloride | 7.5 micrograms |
| Flavin solution | corresponding to 1.6 grams of egg white |
| Cod liver oil | 2 drops |

When vitamin B₁ was subtracted from the above diet rats developed polyneuritis within forty days. The duration until the development of polyneuritis was somewhat longer in the case when purified starch or dextrin was given as the source of carbohydrate than when glucose or sucrose was given. Even the rats nourished on the deficient diet supplemented with daily dose of 1.5 micrograms of vitamin B₁ showed apparent polyneuritic symptoms when glucose or sucrose was fed. From these results it is concluded that rats need more vitamin B₁ when glucose or sucrose is used.

Nearly the same results were also obtained concerning vitamin B₂.

Rats receiving the diet containing butter fat in high percentage, lacking in carbohydrate, developed the syndromes of acrodynia when vitamin B₆ was absent, so also did the rats fed on sucrose diet which contained no fat except linolic acid.

| | Fatty diet | Sucrose diet |
|--------------------------------------|---|--------------|
| Fish protein | 25 | 25 |
| Salts mixture | 5 | 5 |
| Butter fat | 70 | — |
| Sucrose | — | 70 |
| Daily supplements | | |
| Vitamin B ₁ hydrochloride | 7.5 micrograms | |
| Flavin solution | corresponding to 1.6 grams of egg white | |
| Nicotinic acid | 1.5 milligrams | |
| Linolic acid | 50 milligrams | |
| Biosterin (Vitamin A and D) | 2 milligrams weekly | |

In both cases the acrodynia was cured by the addition of crystalline vitamin B₆ hydrochloride at the level of 15 micrograms daily. When linolic acid was subtracted from the above sucrose diet, the animals also developed acrodynia which was completely cured by the single addition of crystalline vitamin B₆, though the growth was not sufficiently improved. These experiments show that vitamin B₆ is an essential factor even for the rats fed on carbohydrate free diet and that fat does not spare vitamin B₆.

Polished rice, thoroughly washed with water and dried, contained a significant amount of vitamin B₆, since the rats fed on the diet, of which the carbohydrate of vitamin B₆ deficient diet was replaced by polished rice, developed no symptom of acrodynia. Commercial rice, corn and potato starches also contained a small amount of vitamin B₆ which was not extracted by simple treatment with alcohol, but was extracted after digestion with such an enzyme as pepsin. Rats receiving the starch purified by the enzymatic digestion followed by alcoholic extraction as the source of carbohydrate developed the same syndromes of acrodynia after four to five weeks as in the case of sucrose feeding.

Isolation of Three Kinds of the Pigment of Flavon Type from Soya Bean.

(pp. 369~372)

By Koji OKANO and Iwao BEPPU.

(The Central Laboratory of South Manchuria Railway Co ;

Received Apr. 8, 1940)

Über die Nutzbarmachung des Vitamins C aus dem Pflanzenreich in Taiwan.

(SS. 372~385)

(I. Mitteilung.) Über die Reingewinnung von Vitamin C
aus Ananassaft mit Hilfe von MgO.

Von Ryo YAMAMOTO und Takeshi HARA.

(Agrikulturchemisches Laboratorium der Taihoku Kaiserlichen Universität, Taiwan ,

Eingegangen am 1. 4. 1940)

Wir haben festgestellt, daß das Vitamin C in Ananassaft mit MgO zu einem unloslichen Verbindungskörper geführt und von diesem Körper unter Zusatz von Saure wieder zu einer Lösung zurückgeführt werden kann. Diese Lösung ist so antiscorbutisch aktiv gegen Meerschweinchen wie die Ascorbinsäurelösung, deren reduzierendes Vermögen gegen 2:6-Dichlorphenolindophenol ganz dasselbe der ersten Lösung ist. Von dieser Lösung isolierten wir ein Ascorbinsäurederivat und nach ihrer Behandlung mit Methanol, Aceton und Aether schieden sich Kristalle ab, welche mit den der Ascorbinsäure übereinstimmen.

(II. Mitteilung.) Über die Konservierung von Ananas-
Vitamin-C in Trockenmilch.

Von Ryo YAMAMOTO und Takeshi HARA.

Trocknet man Milch und Ananassaft nach entsprechender Mischung, so entsteht die charakteristische Trockenmilch, reich an Vitamin C, von einem Gehalt von etwa 50~200 mg %. Nach dem Tierversuche und auch nach der titrimetrischen Methode mit 2:6-Dichlorphenolindophenol zeigt sich, daß der C-Vitamingehalt dieser Trockenmilch fast 100%ig nach Verlauf von 3 Monaten, 95%ig nach 5 Monaten und 90%ig nach 8 Monaten wohl unverändert in Glasflaschen erhalten wird.

(III. Mitteilung) Der Vitamin C Gehalt von nicht eßbaren grünen Blättern aus Taiwan

Von Ryo YAMAMOTO, Takeshi HARA und Shizuko NISHIZAWA.

Wir haben nach der titrimetrischen Methode mit 2 : 6-Dichlorphenolindophenol eine Untersuchung über den C-Vitamingehalt der grünen, nicht eßbaren Blätter aus Taiwan ausgeführt. Die erhaltenen Resultate sind die folgenden : (Für grüne Materialien mg %)

Passiflora edulis, Sims.: 870; *Citrus Limon*, Burm. var. *Ponderosa*, Hort.: 615; *Passiflora laurifolia*, Linn.: 387; *Mangifera indica*, Linn.: 350, *Rennus communis*, Linn.: 275 ect.

Biochemical Studies on "Bakanae" Fungus of the Rice.

Part V. Effect of Gibberellin on Growth, Fermentation and Size of Yeast Cell.

(pp. 385~388)

By Takesi IIAIASI.

(Imperial Agricultural Station, Received Apr. 26, 1940)

Gibberellin, the active principle which makes the rice seedlings grow abnormally tall, has no influence on the growth, fermentation or size of the yeast cell.

Über die quantitative Bestimmung der Pyrethrine.

(SS. 389~410)

VII. Mitteilung. Untersuchung der Bestandteile des Pyrethrumextraktes, die mit den Vorgängen bei der Maßanalyse von Pyrethrinen in irgend einer Beziehung stehen.

Von Sankiti TAKEI, Minoru ŌNO u. Kōkiti NAKASIMA.

(Aus d. Institut f. Chem. Forschung u. d. Agrikulturchem. Laborat. d. Universität, Kyoto, Eingegangen am 26. April, 1940)

Bei der Maßanalyse von Pyrethrin-I und -II muß man außer der Chrysanthemummono- und -dicarbonsäure noch das Vorhandensein einiger stets beigemengter organischer Säuren beachten; trotzdem sie der Menge nach gegenüber den Chrysanthemumsäuren unbedeutend sind, kann sich doch durch sie irgend eine unerwartete experimentelle Fehlerquelle ergeben.

Wir haben in 600 g Pyrethrumextrakt (Pyrethringehalt ca. 20%) gemäß der Behandlung bei der Maßanalyse von Pyrethrinen die untenstehenden Säuren festgestellt:

In den organischen Säuren, die *wasserlösliches* Ba-salz bilden, besteht der

größte Teil (97.74%) aus den beiden Chrysanthemumsäuren (Chrysanthemummonocarbonsäure 46.80% und Chrysanthemumdicarbonsäure 51.44%), der Rest (2.26%) setzt sich zusammen aus Essigsäure (etwa 1/3), iso-Buttersäure, Capronsäure, Caprylsäure und Caprinsäure. Chrysanthemumsäuren finden sich in den wasserunlöslichen Ba salz bildenden Säuren nicht. Aus letzteren konnten wir hauptsächlich Palmitin-, Öl-, Linol-, Linolen-, Behen-, Carnaub- und Azelainsäure gewinnen; außerdem haben wir noch eine kleine Menge Laurin-, Myristinsäure und undefinierte Harzsäure konstatiert.

Auf Grund dieser Ergebnisse läßt sich annehmen, daß bei der Maßanalyse der Pyrethrine durch Baryta-Behandlung der größte Teil der beigemengten organischen Säuren eliminiert wird, so daß der sich bei dieser Methode ergebende experimentelle Fehler tatsächlich sehr gering ist und nicht beachtet zu werden braucht.

VIII. Mitteilung. Stufenweise Untersuchungen zur maßanalytischen Bestimmung des Pyrethrins mittels rein isolierter Chrysanthemummono- und -dicarbonsäure.

Von Sankiti TAKEI u. Kiyosi WAKAZONO.

Ob die Chrysanthemummono- und -dicarbonsäure während des ganzen Prozesses der Maßanalyse ohne Verlust quantitativ bestimmt zu werden vermag, kann man mittels des Pyrethrumextraktes, das viele Beimengungen enthält, nicht exakt untersuchen, man muß vielmehr zu diesem Zweck mit rein isolierter einheitlicher Chrysanthemummono- bzw. -dicarbonsäure arbeiten. Nach wiederholter Reinigung kristallisiert die reine Chrysanthemummonocarbonsäure bei etwa 10°C und schmilzt bei etwa 18~20°. Die reinen Kristalle der Chrysanthemumdicarbonsäure schmelzen bei 164°.

Unsere vorsichtig und wiederholt ausgeführten Untersuchungen über die einzelnen Arbeitsstufen bei der Maßanalyse haben keinen merklichen Verlust an beiden Chrysanthemumsäuren beobachten lassen. Nach einigen weiteren Experimenten vermochten wir die Brauchbarkeit der Maßanalyse-Methode für die Pyrethrin-Bestimmung zu bestätigen.

On the Enclosed and Reclaimed Marsh Soil on the Coast of Kyushu.

(pp. 411~416)

By R. KAWASHIMA and M. NAGATA.

(Agricultural Chemical Laboratory, Kyushu Imperial University; Received Apr. 24, 1940.)

Enzymic Studies on Cereals

(pp. 417~438)

By Gohei YAMAGISHI.

(Morioka Imperial College of Agriculture and Forestry; Received Apr 24, 1940)

(Part XI). On the Adsorption of the Amylase of Rice.

In the studies performed up to this time the author has pointed out that there are three amylases (the starch liquefying, the starch-dextrinifying, and the starch-saccharifying enzymes) in rice.

The present experiment has been carried out to confirm whether these amylases can be separated by treating with the adsorbing agent.

The results obtained from this investigation may be summarized as follows :

(1) Studies were made on the adsorption of the starch-splitting enzymes in the germinated rice. As the adsorbing agent aluminium hydroxide A prepared by Willstatter's method was employed.

(2) It was confirmed that under the author's experimental conditions the adsorption of the enzyme was completed within almost thirty minutes.

(3) The degree of adsorption was increased with the increasing quantity of the adsorbing agent.

(4) It was observed that the higher the temperature (below 50°C.), at which the enzymes were extracted from the sprouted rice, the more the enzymes were adsorbed.

(5) The higher the concentration of the enzymes solution, the greater the degree of adsorption.

(6) The adsorption was influenced with the concentration of the hydrogen ion of the enzyme solution and the optimum pH for adsorption was found to be about 4.0~4.5.

(7) When the concentration of the acetate buffer solution (pH 4.62) was increased over 0.1~0.2 M, the decrease of the adsorption degree was brought about.

(8) In the case of neutral salts the optimum concentration for the adsorption of the amylases existed.

(9) All the inorganic and the organic salts (0.1 N) showed an effect on the adsorption of the amylases, except sulphate.

(10) Whereas glucose and maltose seemed to have some favourable effect upon the enzyme adsorption, starch showed an inhibitory action.

(11) The degree of adsorption was increased in accordance with the increase of the concentration of alcohol, but glycerin acted quite the contrary.

(12) The fact could be confirmed that when the enzyme extract was allowed to stand at lower temperatures, the degree of adsorption of the enzymes was larger than the case immediately after the extraction.

(13) According to the kind of starch-splitting enzyme there were some dif-

ferences as to the adsorption, and yet it will be almost impossible to separate the starch-liquefying, the starch-dextrinifying and the starch-saccharifying enzymes by this means.

(Part XII). On the Elution of the Adsorbed Amylase of Rice.

In paper XI of this series the author has reported on the separation of the germinated rice by adsorption on aluminium hydroxyde.

In this paper I wish to report the results of the experiments performed on the elution of the enzyme which was once adsorbed.

(1) It was deemed that the elution of the enzyme was finished within a very short period.

(2) There was the optimum hydrogen ion concentration for the elution of the enzyme, but the optimum pH differed depending on the nature of the buffer used, i. e., pH 9.3 (borate buffer) and pH 6.5 (phosphate buffer).

(3) When the pH of the solution was kept constant (6.5) using a phosphate buffer, the degree of the elution also increased in accordance with the increase of the concentration of the phosphate mixture.

(4) On releasing the enzyme with the basic substances, such as NaOH, Na_2CO_3 , NaHCO_3 , Na_2HPO_4 , and $(\text{NH}_4)_2\text{HPO}_4$, each has its respective optimum concentration.

(5) The influence of the neutral salts (inorganic and organic) on the elution of the adsorbed enzyme was very different according to the nature of the salts, and sulphate exhibited the most remarkable effect. Thus it was concluded that K_2SO_4 was most suitable as the eluting agent of the adsorbed amylase.

(6) No effect of alcohol and acetone on the elution was observed.

(7) It was known that the unit enzymic activity of the eluate was increased nearly twenty-five times compared with the original enzyme extract.

(8) Although some difference of the degree of elution was noticed among the starch-liquefying, and the starch-dextrinifying, and the starch-saccharifying enzymes, it will be difficult to separate these three amylases by this method.

On the Hydrolysis of Fats and Fatty Acid Esters. (III)

(pp. 439~453)

By Toyoki Ono.

(Chemical Laboratory of the Fish Oil and Fish Meal Association of Japan;

Received Apr. 26, 1940)

Selective Hydrolysis of Mixed Triglycerides and Fish Oils.

(1) The oleic acid radicals in α - and β -oleodistearin are saponified at 30°C with the same velocity in the homogenous system, but are split off more easily than the stearic acid radicals.

Similar hydrolysis was observed in β -laurodistearin, and showed that such selective hydrolysis is more rapidly carried out in the heterogenous system than in the homogenous system.

(2) On the hydrolysis of fish oils by lipase and KOH at lower temperature (-10°C), the higher unsaturated fatty acid radicals are more rapidly split off than lower unsaturated or saturated ones. Through the analytical results of hydrolysis for β -moroctodiolein, I have further proved these facts.

Table 7 shows this explanation of the mechanism of hydrolysis of mixed triglycerides.

Table 7 Selective Hydrolysis of Mixed Triglycerides.

| Glycerides | Method of Hydrolysis | Iodine Value | | | |
|-------------------------|----------------------|---------------|--------|---------------|--------|
| | | I | II | III | |
| α -Oleodistearin | KOH | 38 40 | 33 25 | 34 85 | |
| β -Oleodistearin | KOH | 28 17 | 22 00 | 25 71 | |
| β -Morocodiolein | Lipase | 160 15 | 165 60 | 165 60 | |
| | | Melting Point | | Neutral Value | |
| | | I | III' | I | III' |
| β -Laurodistearin | KOH | 59 | 60 | 224 40 | 217 53 |

I Free fatty acids liberated from glycerides

II Unhydrolysed part,

III Original glycerides

III' Fatty acids in original glycerides

Sterilizing Action of Acids. 12th Report.

Sterilizing Action of Aromatic Acids.

(pp. 454~460)

By Sogo TETSUMOTO

(Government Institute for Infectious Diseases, Tokyo Imperial University,

Received Feb. 27, 1940.)

(1). Concerning the sterilizing action of mineral acids, fatty acids and phenols I already reported.

Now I studied the sterilizing action of aromatic acids and their salts. Aromatic acids and their salts are contained in skins, leaves, flowers, and fruits of various vegetables, so they have an intimate relation to our daily life. Accordingly we see many reports concerning the sterilizing action or preservative power of aromatic acids and their salts on bacteria.

But many of the previous reports are almost limited to the sterilizing action

or preservative power of salicylic acid, benzoic acid, cinnamic acid, tannic acid and their salts, while there are many aromatic acids and salts other than these.

In this experiment the sterilizing action at the same concentration was tested of as many aromatic acids as I could gather, in order to elucidate the relation of the number and position of CO_2H and OH group, pH, salts anion, etc., to the sterilizing action.

(2). Reagents.

Reagents used are listed in Table 1.

The following problems were studied by using these reagents :

- (1). Sterilizing action of aromatic acids at the same concentration.
- (2). The effect of aromatic acid salts and anions on the life of bacteria.
- (3). The effect of the number of CO_2H group and OH group of aromatic acids on the sterilizing action of bacteria.

Table I. Aromatic acids.

| Number of CO_2H group | Number of OH group | Acid | Rational formulae | M W | Weight % at N/1000 |
|---------------------------------------|-----------------------------|----------------|---|----------|--------------------|
| 1 | 0 | Benzoic | $\text{C}_6\text{H}_5 \cdot \text{CO}_2\text{H}$ | 122 048 | 0 0122 |
| | 1 | Salicylic | $\text{C}_6\text{H}_4 \begin{matrix} \nearrow \text{CO}_2\text{H} \text{ (1)} \\ \searrow \text{OH} \text{ (2)} \end{matrix}$ | 138 048 | 0 0138 |
| | 0 | Cinnamic | $\text{C}_6\text{H}_5\text{CH}=\text{CH} \cdot \text{CO}_2\text{H}$ | 148 064 | 0 0148 |
| | 1 | Mandelic (1) | $\text{C}_6\text{H}_5 \cdot \text{CHOH} \cdot \text{CO}_2\text{H}$ | 152 064 | 0 0152 |
| | 2 | Protocatechuic | $\text{C}_6\text{H}_3 \begin{matrix} \nearrow \text{CO}_2\text{H} \text{ (1)} \\ \searrow \text{OH} \text{ (3) + H}_2\text{O} \text{ (4)} \end{matrix}$ | 172 099 | 0 0172 |
| | 3 | Gallic | $\text{C}_6\text{H}_2(\text{OH})_3 \cdot \text{CO}_2\text{H}$ (3), (4), (5) | 184 048 | 0 0184 |
| | 4 | Chinic | $\text{C}_6\text{H}_7(\text{OH})_4 \cdot \text{CO}_2\text{H} + \text{H}_2\text{O}$ | 210 112 | 0 0210 |
| 2 | | Tannic | $\text{C}_{11}\text{H}_{10}\text{O}_7 + 2\text{H}_2\text{O}$ | 358 112 | 0 0358 |
| | 0 | Phthalic (nor) | $\text{C}_6\text{H}_4 \begin{matrix} \nearrow \text{CO}_2\text{H} \text{ (1)} \\ \searrow \text{CO}_2\text{H} \text{ (2)} \end{matrix}$ | 166 048 | 0 0083 |
| 3 | 0 | Trimellitic | $\text{C}_6\text{H}_2 \begin{matrix} \nearrow \text{CO}_2\text{H} \\ \searrow \text{CO}_2\text{H} + \text{H}_2\text{O} \\ \text{CO}_2\text{H} \end{matrix}$ | 228 109 | 0 0076 |
| 6 | 0 | Meillic | $\text{C}_6(\text{CO}_2\text{H})_6$ | 342 108 | 0 0057 |
| | | Salphanilic | $\text{C}_6\text{H}_4 \begin{matrix} \nearrow \text{SO}_3\text{H} \\ \searrow \text{NH}_4 \end{matrix}$ | 173 126* | 0 0173 |

SUMMARY.

By studies on the sterilizing action of aromatic acids and their salts on bacteria I obtained the results, the summary of which is as follows:—

- (1). The order of strength of the sterilizing action of aromatic acids is :

salicylic acid > salphanilic acid \rightleftharpoons mellitic acid \rightleftharpoons mandelic acid >
 cinnamic acid > protocatechnic acid \rightleftharpoons gallic acid.

Note :— \rightleftharpoons means nearly equal.

The weakest of all are phthalic acid and chinic acid.

(2). Aromatic acids of low pH have stronger germicidal action than acids of high pH like general ordinary acids.

(3). Salts of cinnamic acid, mandelic acid and chinic acid have strong promoting action for the bacteria tested, except *Vib. cholerae*.

(4). Anions of gallic acid, tannic acid and salicylic acid have sterilizing action but other anions except those just mentioned have no sterilizing action.

(5). According to the increasing number of CO₂H group, the sterilizing action of aromatic acids also increases proportionally, but on the contrary there seems to exist the inverse proportion between the increasing number of OH group and the strength of the sterilizing action of aromatic acids. These facts are evidently different from the action of aliphatic acids.

(6). When we compare the strength of the sterilizing action at the same concentration, we see that aromatic acids such as salicylic acid, salphanilic acid, mellitic acid, etc., have stronger sterilizing action than strong mineral acids such as nitric acid, hydrochloric acid and sulphuric acid.

(7). Aromatic acids have no such violent strong sterilizing action as certain few mineral acids or fatty acids, neither do they have so weak bactericidal power or rather promoting power for bacterial life like higher fatty acids such as palmitic acid or stearic acid.

A New Simple Method for the Quantitative Determination of Glycerine.

(pp. 461~475)

By Hogai KA.

(The Institute of Scientific Research, Manchoukuo, Received Apr 10, 1940)

For the development of a quantitative method based on Denigés' glycerine and codein colour reaction, investigations were carried out to determine the conditions for the reactions, such as the colour reactions of the impurities and their elimination, its application and the comparison with other methods, and the results are reported as follows.

1. Solutions of glycerine (below 2.5%) of different purities were prepared and the time of oxidation and the time for the driving out of the excess bromine after the oxidation of the glycerine solutions by 0.4% bromine water, and the time of warming after the addition of codein solution and H₂SO₄ were investigated and the following results were obtained :

a) The intensity of blue colour increased with an increase of the time of oxidation but became nearly constant after 25 minutes.

b) The time of driving off the excess bromine had no distinct influence on the blue-colour value.

c) The intensity of the blue colour value increased with an increase of the time of warming after the addition of codein and H_2SO_4 and reached constant after 20 minutes. Consequently, the suitable conditions for the Br_2 oxidation and the time of warming after the addition of codein and H_2SO_4 were 25 and 20 minutes respectively.

2. 5 cc amounts of the glycerine solution of each different concentration were taken and were oxidised with the addition of 10 cc, 20 cc and 30 cc of 0.4% Br-water and 20 cc of 0.8% Br-water respectively under the conditions named above, and it was found that the intensity of the blue-colour value was maximum when enough Br_2 was added while the addition of excess quantities of Br_2 caused no change in the colour value.

Further, it was found that the maximum blue-colour value was in direct proportion to the concentration of glycerine. Therefore the determination between codein and glycerine which has been previously oxidised with an excess of Br_2 with Lovibond's Tintometer, can be calculated from the following equation :

$$\text{Glycerine \%} = 0.04646 (\text{blue value} - 1.5754)$$

3. The presence of H_2SO_4 and Na_2SO_4 showed no change in the blue value.

4. Attempts were made to eliminate the impurities by using lime, sugars, aldehydes, acids, alcohols, etc., which were considered to interfere with the blue value. With the exception of alcohols, all of the impurities could be eliminated.

5. The quantities of impurities in the glycerine of fats and oils, especially plant oils, are so small that glycerine can be determined directly after their saponification and decomposition with dilute sulphuric acid. This method gave approximately the same value as that of the acetin method, but the dichromate method gave a somewhat higher value.

6. Approximately the same values were obtained in the quantitative determinations of glycerine of known concentrations by this method after the elimination with lime of the impurities which were added experimentally.

Judging from the foregoing results, this method can be applied for the quantitative determination of glycerine in soy or wine after the elimination of impurities with lime.

Studies on the Tannin of *Acacia confusa* Merrill (I).

On the Nature of Tannin and Catechin.

(pp. 476~478)

By Minoru ISII and Yasuyosi ŌSIMA.

(Agricultural Chemical Department, Taihoku Imp University, Taiwan;

Received Apr 15, 1940)

Acacia confusa Merrill (Japanese name Sosiju) is a chief fuel material in Taiwan, the bark of which contains about 10% of tannin substances. The purified tannin is a light brown amorphous powder and by chemical researches it was decided as a phlobaphene producing tannin. It shows specific rotation $+70.8^\circ$ and gives 5.07% H and 59.51% C on analysis. We separated two catechins from the catechin mixture which amounted to 0.12% of the bark and each were confirmed as *d*-catechin and *l*-epicatechin by the following data.

| | m p | Specific rotation | $[\alpha]_D$ | H% | C% |
|------------------------|------|-------------------|---------------|------|-------|
| <i>d</i> -Catechin | 176° | $\pm 0^{(1)}$ | $+16.7^{(2)}$ | 4.78 | 62.12 |
| <i>d</i> -Pentaacetate | 133° | $+40.8^{(3)}$ | | 4.83 | 60.35 |
| <i>l</i> -Epicatechin | 237° | $-65.0^{(1)}$ | | 4.84 | 62.10 |
| <i>l</i> -Pentaacetate | 153° | $-13.0^{(3)}$ | | 5.10 | 60.14 |

(1) 98% ethylalcohol (2) 50% acetone (3) acetylenetetrahydroxide

Studies on Ascorbic Acid. II.

The relation between glycolysis, ascorbic acid and glutathione in the defibrinated blood of the healthy rabbit.

(pp. 479~492)

By Kichinosuke FUJIMURA.

(Chemical Institute, Kyoto Imperial University, Received Apr 1, 1940)

In the course of glycolysis in the defibrinated blood of the healthy rabbit, the following relations between blood sugar and ascorbic acid were found.

1 The glycolysis in the defibrinated blood of the healthy rabbit was complete in about 4 hours.

2 The glycolysis in the defibrinated blood of the healthy rabbit to which was added the ascorbic acid oxidase was completed later than the glycolysis in the defibrinated blood to which no ascorbic acid-oxidase was added, and the content of the reduced ascorbic acid in it was about equal to that which included no ascorbic acid oxidase.

3 The glycolysis in the defibrinated blood of the healthy rabbit to which was added the reduced ascorbic acid was completed earlier than that which included no reduced ascorbic acid, in proportion to the quantity of the reduced ascorbic acid added.

4 Therefore, as to the glycolysis in the defibrinated blood of the healthy rabbit the reduced ascorbic acid plays an important role in the oxidation reduction system and the reduced glutathione protects the reduced ascorbic acid from oxidation.

Studies on Vitamin C. (IV)

On the Isolation of Ascorbic Acid.

(pp. 493~500)

By Hisateru MIYUDA

(Laboratory of Nutritional Chemistry, Kyoto Imperial University)

Received Apr 1 1940)

With special consideration of the fact that vitamin C is very unstable to oxidation but extremely stable to heating, I have tried to isolate ascorbic acid by studying the following points

- 1) The effect of steaming on natural products containing ascorbic acid.
- 2) Suitable solvents
- 3) The optimum pH of fuller's earth when used in the elimination of colouring matter
- 4) The use of mercuric acetate in purifying ascorbic acid

In addition some interesting suggestions relative to the substance of ascorbic acid oxidase were discovered

I take this opportunity to express my sincere thanks to Prof K. Kondo for his sympathetic guidance and encouragement throughout the course of these studies
(March 19, 1940.)

Correction.

Zirō HIROSE: On the Denaturation of Sericin (Part 1)

Vol. 16, No. 3; March, 1940.

On page 44, line 32, read " 8 139 g/l " for " 1 139 g/l "

N. B.—Inadvertently the following plates were omitted from the previous issue. To be inserted between p 68 and p 69, This Bulletin, Vol 16, No 4, April, 1940

Explanation of Plates.

Spectra showing transmission wave lengths through the filters

Plate I. (Ilford Infra-red plate)



Plate II (Kodak Panatomic plate)

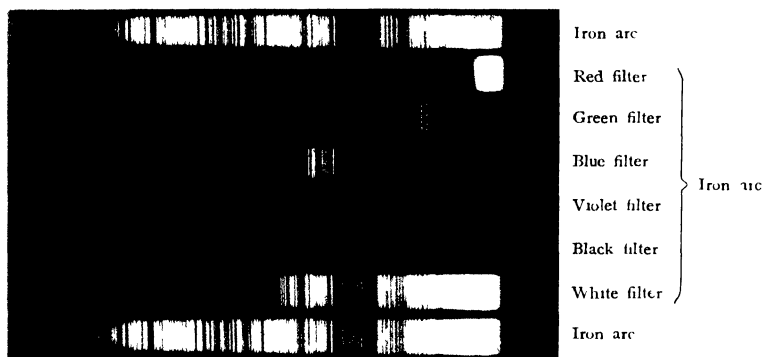


Plate III (Oriental Hypersensitive Panchromatic plate)

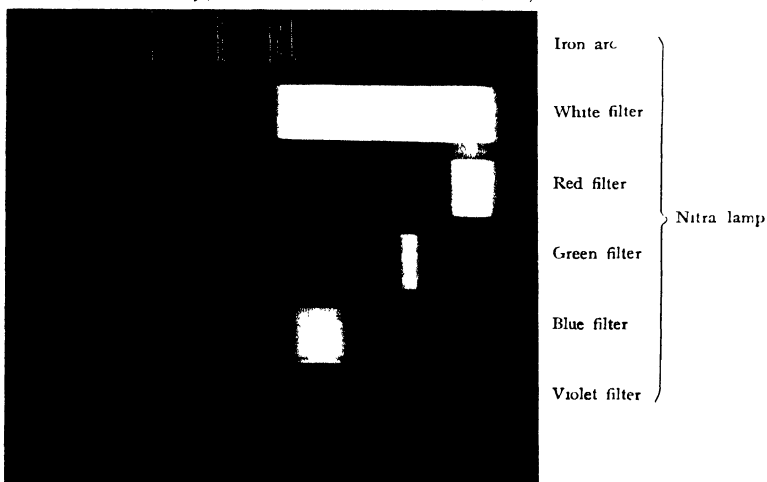
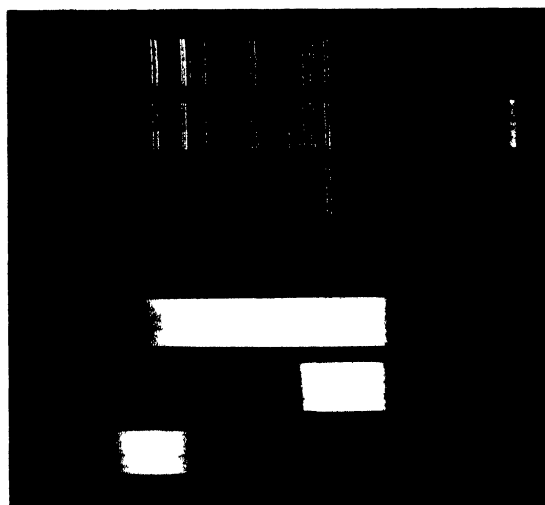
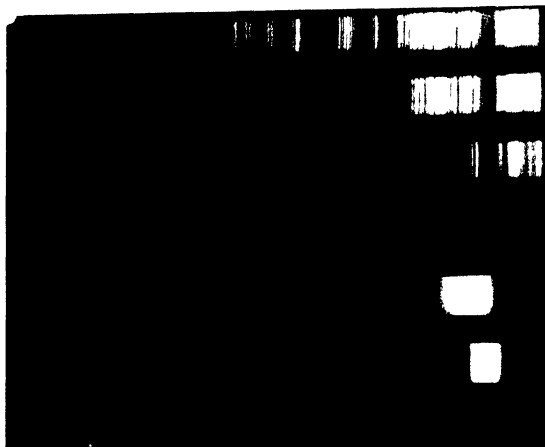


Plate IV. (Oriental Hypersensitive Panchromatic plate)



| | | |
|------------------------------|---|-------------------------|
| Iron arc | } | Iron arc. |
| White filter | | |
| Ultra-violet close filter | } | "Vim Ray" blue lamp. |
| Ultra-violet pass filter | | |
| White filter | | |
| Ultra-violet close filter | | |
| Ultra-violet pass filter | | |

Plate V. (Oriental Hypersensitive Panchromatic plate)



| | | |
|------------------------------|---|-------------------------|
| Iron arc | } | Iron arc. |
| White filter | | |
| Ultra-violet close filter | } | "Vim Ray" blue lamp. |
| Ultra-violet pass filter | | |
| White filter | | |
| Ultra-violet close filter | | |
| Ultra-violet pass filter | | |

Plate VI. (Ilford Panchromatic plate)

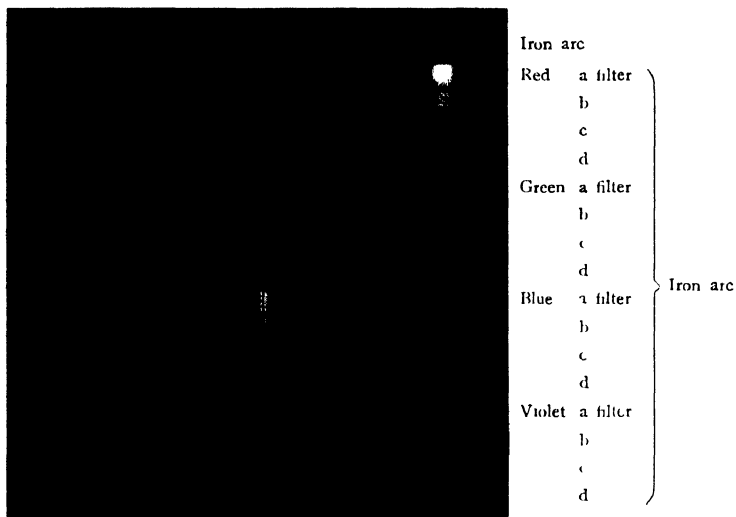
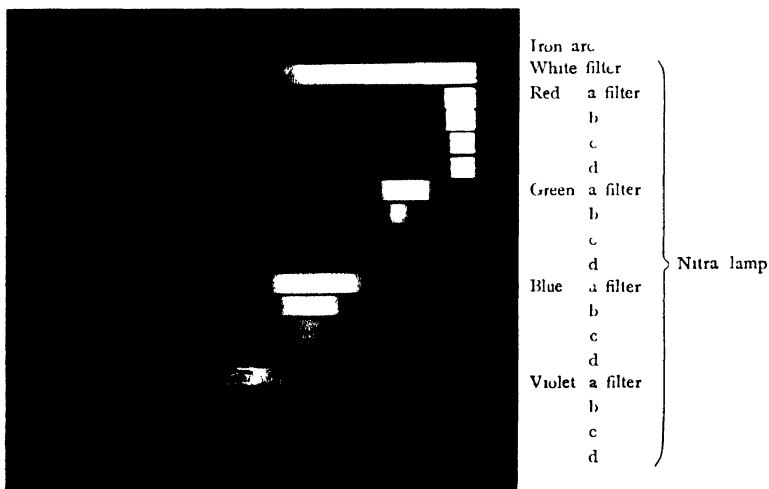


Plate VII. (Oriental Hypersensitive Panchromatic plate)



Bulletin of the Agricultural Chemical Society of Japan.

TRANSACTIONS

Biochemistry of Filamentous Fungi. VI.

Mycelial Constituents of *Oospora sulphurea-ochracea*. Part III. Trimethylsulochrin and its Fission Products.

By Hidejiro NISHIKAWA.

(Tottori Agricultural College.)

Received May 11, 1940

Of the crystalline constituents of the mycelium of *Oospora sulphurea-ochracea* described in the previous communication,⁽¹⁾ sulochrin (substance B) was proved by further experiments to be methyl ester of 2:6:4'-trihydroxy-4-methyl-6'-methoxy-benzophenone-2'-carboxylic acid, the structural discussion having been advanced in detail in a separate paper.⁽²⁾

Of the three hydroxyl groups which are present in the sulochrin molecule two could be readily methylated by means of diazomethane; one of the two hydroxyl groups in the *p*-orsellinic moiety situated at ortho-position to the central carbonyl resisted this means of methylation.

The fully methylated derivative has now been prepared by repeated application of dimethylsulphate on sulochrin. Trimethylsulochrin thus obtained can be split neatly into two halves by means of conc. sulphuric acid followed by the addition of water, just as in the case of sulochrin and dimethylsulochrin, resulting fragments being, as is anticipated, dimethyl-*p*-orsellinic acid and methyl dimethyl- α -resorcyate and thus giving an additional evidence of the structure of sulochrin. A more drastic measure of hydrolysis, however, is required in this case. While in the case of sulochrin and dimethylsulochrin extreme decomposition had to be controlled by ice-cooling the mixture, for the complete hydrolysis of trimethylsulochrin it was necessary to warm the sulphuric acid solution for some time on a water-bath.

Action of methyl alcoholic potash on trimethylsulochrin yields monomethyl-tetramethoxy-benzophenonetricarboxylic acid which is isomeric with dimethylsulochrin, one methoxyl of ester-form having been lost.

EXPERIMENTAL.

Trimethylsulochrin (4-methyl-2 : 6 : 4' : 8'-tetramethoxy-2'-carbomethoxybenzophenone).

Five grams of sulochrin was dissolved in 12.5 cc of 10% NaOH and shaken vigorously on a machine, 15 cc of dimethyl sulphate and 77.5 cc of 10% NaOH being alternately dropped in during three hours. Amorphous solids separated and were collected, yield nearly quantitative. One gram of the crude material, which contained incompletely methylated impurities, was dissolved in acetone, 2 cc of dimethyl sulphate was added and, while shaking, 12 cc of 10 % NaOH was added drop by drop. To the resulting clear solution much water was added till the precipitation of crystals occurred (yield nearly quantitative) which melted at 152°. On recrystallization from benzene the substance crystallized in platelets and melted at 157°, turning pink. The melting-point was quite similar to that of dimethylsulochrin and to make sure of their dissimilarity a mixed melting-point was taken which showed marked depression. (Found: C, 64.40; H, 5.94%. $C_{18}H_{20}O$ requires C, 64.17; H, 5.88%. Methoxyl. Found: 40.93%. $5(CH_3O)$ in $C_{18}H_{20}O$ requires 41.44%.)

Trimethylsulochrin dissolves readily in acetone, chloroform, methyl alcohol, ethyl acetate, moderately in ethyl alcohol and benzene, slightly in ether, but not in light petroleum and water. It does not give a $FeCl_3$ reaction.

Fission of trimethylsulochrin by means of conc. sulphuric acid.

To 2.4 g of trimethylsulochrin was added 24 cc of conc. sulphuric acid and the mixture was warmed on a water-bath until the initial dark brown colour of the solution changed into a deep reddish purple tint. The whole matter was then poured into a large amount of water, the resulting emulsion being thoroughly extracted with ether. The ether layer was shaken twice with 5% bicarbonate solution, this in turn was acidified and again extracted with ether. The ether solution was dried and distilled, 0.7 g substance being left behind. For purification it was dissolved in methyl alcohol and precipitated by the addition of water. It melted at about 165° and gave a green $FeCl_3$ reaction. The crystals were again dissolved in a small quantity of warm methyl alcohol and cooled with ice, when beautiful square platelets devoid of $FeCl_3$ reaction separated out. The melting point now rose to 182° and mixed melting point with a synthetic specimen of dimethyl-p-orsellinic acid showed no depression.

The fraction insoluble in bicarbonate, after distilling off the solvent, was left behind as a yellowish oil (0.7 g), in which was seeded a tiny fragment of methyl dimethyl- α -resorcyate. A magma of large needle crystals separated which melted at 44° alone or mixed with an authentic specimen of methyl- α -resorcyate.

4-Methyl-2 : 6 : 4' : 6'-tetramethoxy-2'-carboxybenzophenone.

Trimethylsulochrin (1 g) was boiled with methyl alcohol (30 cc) and KOH (1 g) under reflux for five hours. On cooling it was diluted with water and acidified with HCl, when the liquid became turbid and gradually separated prism crystals (0.75 g). It melts at 194° and gives neither FeCl_3 nor CaOCl_2 reaction. (Found: C, 63.04; H, 5.61%. $\text{C}_{19}\text{H}_{20}\text{O}_7$ requires C, 63.33; H, 5.56%. Methoxyl. Found: 34.11%. $4(\text{CH}_3\text{O})$ in $\text{C}_{19}\text{H}_{20}\text{O}_7$ requires 34.44%). It readily dissolves in acetone, methyl alcohol, chloroform, moderately in ethyl alcohol, less in ethyl acetate and benzene, sparingly or not in light petroleum, ether and water.

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- (1) Nishikawa. Bull. Agr. Chem. Soc. Jap., 13, 1 (1937)
- (2) Nishikawa: Acta Phytochim., 11, 167 (1939)

ABSTRACTS

from

TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noted.)

On the Stimulant for Cane Sugar Formation in Plants. (VI)

(pp. 501~503)

By Tetutara TADOKORO and Masao NISIDA.

(Hokkaido Imperial University, Received May 14, 1940)

Separation and Identification of Fatty Acids.

(pp. 504~512)

By Y. INOUE and H. YUKAWA.

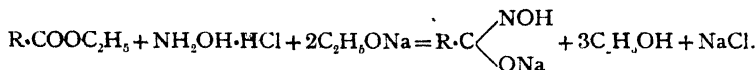
(Agr. Chem Laboratory, Kyoto Imp. Univ., Received May 9, 1940)

Part I. Hydroxamic Acids derived from Saturated Fatty Acids.

For making a research of the chemical structures of fats and oils or their fatty acids, we must establish the methods for the pure separation of each acid. At least it is requisite to isolate each fatty acid in the crystal state.

Hydroxamic acids which can be obtained from fatty acids by reaction with hydroxylamine are crystal substances with relatively higher melting points. Therefore, we have made the fundamental experiments utilizing these properties.

Although there were several methods used to obtain hydroxamic acid, we studied the reaction of esters or glycerides with hydroxylamine in the presence of sodium ethylate in order to prepare it directly from fats and oils. The reaction was as follows:



This reaction proceeded quantitatively at room temperature without moisture.

Hydroxamic acids derived from saturated fatty acids which were obtained in the form of white crystals gave an intensive reddish-violet colour in alcohol with ferric chloride and gave green, voluminous and amorphous precipitation of copper salt in an excess alcoholic solution of copper acetate. And also we could recover the original fatty acid by refluxing the hydroxamic acid with diluted alcoholic

solution of sulphuric acid. The solubility was different in several organic solvents according to the carbon numbers of hydroxamic acids.

Table. Melting points and solubilities of hydroxamic acids.

| Fatty acid | Fatty acid M P °C | M P °C | Hydroxamic acid | | | | |
|-----------------|----------------------|-----------|-----------------|--------|-------|-------|------------|
| | | | Solubility | | | | |
| | | | Ethanol | Aceton | Ether | Water | Petr-ether |
| C ₂₂ | 84 | 112.5 | + | + | - | - | - |
| C ₂₀ | 77 | 109.5~110 | + | + | - | - | - |
| C ₁₈ | 71.5~72 | 106.5~107 | + | + | - | - | - |
| C ₁₆ | 63.5~64 | 102.5 | + | + | - | - | - |
| C ₁₄ | 57.5~58 | 98~98.5 | + | + | + | - | - |
| C ₁₂ | 47.5~48 | 94 | + | + | + | - | - |
| C ₁₀ | 31.5 | 88~88.5 | + | + | + | + | - |
| C ₈ | 16 | 78.5~79 | + | + | + | + | - |
| C ₆ | -1.5 | 63.5~64 | + | + | + | + | - |
| C ₄ | -4.7 | syrup | + | + | + | + | - |
| C ₃ | -19.7 | 92.5~93 | + | + | + | + | - |
| C ₂ | 16.7 | 88 | + | + | + | + | - |

The increasing number of (+) means greater solubility and the (-) insoluble.

Part II. Hydroxamic Acids derived from Unsaturated Fatty Acids.

In the previous work we derived hydroxamic acids from saturated fatty acids which contained an even number of carbon atoms, $C \sim C_{2n}$, and decided their melting points. In the present work we obtained olein-, linol-, and linolenhydroxamic acids in the form of white crystals by the reaction of hydroxylamine to their ethyl esters in the presence of sodium ethylate, as before.

These were soluble in organic solvents, possible to recrystallize from petroleum ether, gave an intensive reddish-violet colour in alcohol with ferric chloride and gave green, voluminous, amorphous precipitation of copper salt with an excess alcoholic solution of copper acetate. Also the original fatty acid could be recovered by means of refluxing the hydroxamic acid with diluted alcoholic solution of sulphuric acid. Their melting points were 61°, 41~42° and 37~38°. The inclination of solubility in petroleum ether was olein- < linol- < linolenhydroxamic acid.

On the Teratologic Forms of *Aspergillus* *Awamori* var. *fumeus*.

(pp. 513~518)

By MATAZO ABE.

(Scientific Laboratory of Ch. Takeda & Co. Ltd., Osaka, Received May 15, 1940)

During the morphological studies of *Asp. Awamori* var. *fumeus* Nak., Sim. et Wat. the following facts have been observed

- 1). A "white wooliness" of the fungus originates from its mycelium and sterile hyphae.
- 2). Abnormal conidiophores are produced from this mycelium.
- 3). Sterile hyphae are formed by the proliferation of some of the secondary sterigmata.
- 4). Some normal conidiophores bear a sort of abnormal forms growing parasitically in the stalks and the vesicles.
- 5). The greater part of these parasitic forms grow out into abnormal conidiophores which, in turn, produce conidia both within and without the "host"

Studies on the Nutritive Value of Weeds.

(pp. 519~527)

By GÔITI FUKAI

(Agricultural Chemical Laboratory, Tokyo Imperial University,
Received May 21, 1940)

I. Carotene and Vitamin C Contents and Their Fluctuations.

II. Vitamin B₁ and B₂ Contents.

Nutritional Conditions of the Wild Grazing Horse.

(pp. 528~530)

By GÔITI FUKAI.

(Agricultural Chemical Laboratory, Tokyo Imperial University,
Received May 21, 1940)

Biochemical Studies on "Bakanae" Fungus of Rice. Part VI.

Effect of gibberellin on the activity of amylase in germinated cereal grains.

(pp. 531~538)

By Takesi HAYASI.

Imperial Agricultural Station; Received May 8, 1940)

Gibberellin, the active principle which makes the rice seedlings grow abnormally tall, has stimulative action on the germination of barley (hulled and naked), wheat and rice grains and on the activity of amylase in germinated barley (hulled and naked) and wheat grains.

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Studies on the Fibres of the Skinfat-layers of the Whale.

(pp. 539~540)

By Tosio NAKAHAMA and Masao HASEGAWA.

(Kobe Yamashina Institute, Received May 21, 1940)

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Über die Verwitterung der Eruptivgesteine. VII.

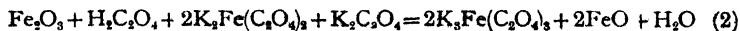
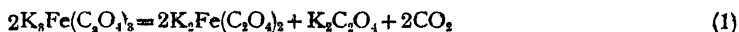
Eine neue Methode zur Bestimmung des freien Eisenoxydes.

(SS. 511~551)

Von Mituru HARADA.

(Landwirtschaftliche Hochschule Tottori, Eingegangen am 20. 5. 1940)

Wie der Verf. in der Mitteilung II und IV berichtet hat, lost sich limonitisches und hamatisches Eisenoxyd in einer schwach oxalsauren Kaliumoxalatlösung unter der Wirkung des Lichts nach einigen Stunden plötzlich auf. Der Chemismus dieser Reaktion liegt darin, dass sich zunächst eine sehr kleine Menge des Eisenoxydes lost und $K_3Fe(C_2O_4)_3$ entsteht, das sich dann photochemisch nach der Gleichung (1) in $K_2Fe(C_2O_4)_2$ umwandelt. Als dann erfolgt die Auflösung des Eisenoxydes mit großer Reaktionsgeschwindigkeit unter der katalytischen Wirkung des $K_2Fe(C_2O_4)_2$ nach dem Schema (2) und (3). Diese Reaktionen gehen unter der Einwirkung der violetten und der ultravioletten Strahlen vor sich; beim Erwärmen findet die Reaktion (2) und (3) auch im Dunkeln statt.



Auf diesen Reaktionen hat der Verf. eine neue Methode zur Bestimmung des freien Eisenoxydes und der Trennung des hamatischen und des limonitischen Eisenoxydes aufgebaut.

REAGENZIEN.

- (1) Oxalsaure-Kaliumoxalatlösung I: 0,025 g-Mol Oxalsaure und 0.1 g-Mol Kaliumoxalat werden zu 1000 ccm gelöst.
- (2) Oxalsaure-Kaliumoxalatlösung II: 0,005 g-Mol Oxalsaure und 0,015 g-Mol Oxalsaure und 0,015 g-Mol Kaliumoxalat werden zu 1000 ccm gelöst.
- (3) Ammonium-Ferrosulphatlösung: 17,5 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 9 g $(\text{NH}_4)_2\text{SO}_4$ und 10 cc $n/10 \text{ H}_2\text{SO}_4$ werden zu 250 ccm gelöst.
- (4) 1%ige Ammoniumchloridlösung.

a) Bestimmung des freien Eisenoxydes:

0,2~1 g gepulverter Probe wird in einem Becherglas mit 250 cc Lösung I übergossen. Das Becherglas wird auf dem siedenden Wasserbade bis auf $80\sim 90^\circ$ erhitzt. Darauf setzt man unter Umrühren 5 cc Ammonium-Ferrosulphatlösung hinzu und erhitzt noch ungefähr 10 Minuten. Nach Zusatz von 2,5 g Ammoniumchlorid läßt man auf Zimmertemperatur abkühlen, filtriert und wäscht mit der Ammoniumchloridlösung aus. Das Eisenoxyd (F_I) im Filtrat wird bestimmt.

Das freie Eisenoxyd in der Probe (E)= F_I -A

(A ist Fe_2O_3 in 5 cc der Ammonium-Ferrosulphatlösung),

b) Bestimmung des nicht limonitischen und nicht hamatischen freien Eisenoxydes:

1 g Probe wird im Dunkeln mit 250 cc Lösung I bei Zimmertemperatur 1 Stunde lange ausgeschüttelt, nach Zusatz von 2,5 g Ammoniumchlorid über Nacht stehen gelassen, und das gelöste Eisenoxyd (F_{II}) wird bestimmt.

c) Bestimmung des limonitischen Eisenoxydes:

0,5~1 g Probe wird in einem Becherglas mit 1 Lösung II übergossen, das Becherglas wird im Dunkeln bis auf 50° erwärmt, hierauf setzt man 5 cc Ammonium-Ferrosulphatlösung hinzu und erwärmt noch 20 Minuten bis auf 50° . Nach Zusatz von 10 g Ammoniumchlorid filtriert und bestimmt man das Eisenoxyd (F_{III}) im Filtrat.

Limonitisches Eisenoxyd (E_I)= F_{III} - F_{II} -A

d) Ausrechnung des hamatischen Eisenoxydes:

Das hamatische Eisenoxyd (E_h)= E - E_I - F_{II} = F_I - F_{III}

Die Bestimmung des freien limonitischen und hamatischen Eisenoxydes in

verschiedenen Bodenarten zeigt, daß sich das Eisen darin größtenteils (80~99% des in konz. Salzsäure löslichen Eisenoxydes) im freien Zustande befindet.

Effects of Certain Mineral Matters on the Growth of Root Nodule Bacteria. (Part III)

(pp. 552~560)

By K. KONISHI, A. KAWAMURA and A. IMANISHI.

(Institute of Agr. Chem., Imp. University, Kyoto, Received May 7, 1940)

Further experiments were conducted to ascertain the effects of chromium and manganese upon *Rh. meliloti*, by measuring Q_{O_2} and R. Q. in both nitrate mannitol solution and phosphate buffer. At the concentration of 0.001 or 0.01 per cent, sulphate of chromium exerted beneficial effects on the oxygen uptake by the organisms and also on their respiratory quotients, while sulphate of manganese did not.

Stimulating action of Cr-sulphate was remarkable as shown by early growth of the organisms on the mannitol media, where nitrogen was supplied with $(NH_4)_2 \cdot SO_4$ as well as $NaNO_3$ in different concentrations. Furthermore, the effect of Cr-sulphate was evident, when sucrose, succinic acid or acetic acid was used as the carbon source.

Über die Technische Citronensäuregarung. II. Mitteilung.

(SS. 561~572)

Von M. NAKANO und K. KOBAYASHI.

(The Institute of Research on Chemical Industry, Government-General of Taiwan, Japan,
Received May 6, 1940)

On the Absorption-Spectrum of Nucleotide.

(pp. 573~574)

By Tetutarō TADOKORO and Naomoto TAKASUGI.

(Hokkaido Imperial University, Received May 6, 1940)

Report on the Shyoty in Tyosen.

(pp. 575~580)

By Y. OHARA.

(Brewing Laboratory, Government General of Tyosen; Received May 16, 1940)

The chemical constitution of 183 specimens of "Kurokozi-, Kyokusi- and Kasutori-Shoty" was investigated. Among these "Kurokozi-Shoty" (Kaoliang, millet, rice, etc, are fermented for 1~2 weeks by "Kurokozi," *Asp. niger* and distilled) is now most usual in North Tyosen.

The following are the results of analysis.

mg in 100 cc Syoty

| Raw material | | Alcohol (vol %) | Acid =acetic | Ester =ethyl | Furfural | Fusel oil | Aldehyde =aceto |
|-----------------|----------------|--------------------|-----------------|-----------------|----------|-----------|--------------------|
| "Kuro- zoki" | Kaoliang (52) | 30.7 | 28 | 33 | 0.2 | 125 | 4.2 |
| | Millet (17) | 31.0 | 20 | 39 | 0.4 | 100 | 3.7 |
| | Rice (11) | 34.0 | 18 | 45 | 1.1 | 148 | 3.3 |
| | "Kyokusi" (10) | 34.0 | 27 | 82 | 1.3 | 58 | 6.5 |
| | "Kasutori" (3) | 33.6 | 31 | 137 | 1.5 | — | 6.1 |

Über die Jodometrie an Furfurol.

(SS. 581~585)

Von Matukitiro HAMADA und Kazuyuki MAEKAWA.

(Aus dem Agrikulturchemischen Institut der Kaiserlichen Kyushu-Universität
in Fukuoka, Eingegangen am 18. 5. 1940.)

Researches on Mechanical Wood Pulp.

(pp. 586~612)

Part II. On a Laboratory Miniature Grinder.

By Mamoru WATANABE.

(Kyoto Imperial University; Received May 5, 1940.)

Part III. On a Classifier for Wood Pulp.

By M. WATANABE, Takesi YASUDA, Kazuaki KAWASE
and Yosifugu KIMURA.

**Part IV. On Howan Howasun (*Larix dahurica* Turcz) of Manchoukuo
as the Raw Material for Ground Wood Pulp.**

By M. WATANABE, T. YASUDA, K. KAWASE and Y. KIMURA.

**Part V. On Yulin Sun (*Picea jezoensis* Carriere) of Manchoukuo
as the Raw Material for Ground Wood Pulp.**

By M. WATANABE, T. YASUDA, K. KAWASE and Y. KIMURA.

**Part VI. On Akamatsu (*Pinus densiflora* S. et Z.) of Nippon
as the Raw Material for Ground Wood Pulp.**

By M. WATANABE, T. YASUDA, K. KAWASE and Y. KIMURA.

Chemical Studies on the Kikyo-root. (Report X)

On the constitutional formulae of platycodigenin. (No. 3)

On the properties of a double bond and the
oxygen atoms of platycodigenin.

(pp. 613~620)

By Magosaburo TSUJIMOTO.

(Agr. Chem. Laboratory, Kagoshima Imp. College of Agr. and Forestry,
Received April 30, 1940.)

SUMMARY.

(1) Platycodigenin reduces an alkaline potassium permanganate solution, readily combines with bromine and iodine, gives a yellow colouration with tetra-nitromethane. Catalytic reduction was unsuccessful. Therefore platycodigenin has a double bond, but it is very inactive.

(2) Platycodigenin possesses seven atoms of oxygen, two of them represented

by -COOH , and four of the others by $(\text{OH})_4$, and one atom of the last still unknown.

(3) From this view; Platycodigenin may be represented by the formulae $\text{C}_{27}\text{H}_{48}\text{O}(\text{OH})_4 \cdot \text{COOH} \cdot \text{F}_1$.

Bulletin of the Agricultural Chemical Society of Japan.

TRANSACTIONS

Oxidation of Tea Tannin by the Action of Oxidizing Enzymes in Fresh Tea-Leaf.

By Yasuyosi OSIMA and Kaneo HAYASI.

(Agricultural Chemical Department, Taihoku Imperial University, Taiwan.)

Received May 27, 1940.

In Taiwan, the manufacturing of black tea has become prosperous year after year and its progress is very remarkable. However, we must acknowledge the superiority of the tea made in India and Ceylon to that which is made in Taiwan. The quality of black tea is due to its colour, taste and flavour in the liquors. In the process of manufacturing, that is, in the operation of withering, rolling and fermentation, tannin is oxidized by enzyme and it is said that the unique colour, flavour and taste are thus produced. We intend to contribute to the improvement of the quality of black tea from both sides by studying tannin and enzyme, which are the chief ingredients of raw tea-leaves.

About the tannin of tea leaves, several reports⁽¹⁾ have been published up to the present time. One of the writers, Osima⁽²⁾ has made a report about the tannin of Taiwan raw tea leaves. There are the reports by Mann and Aso about the enzyme of tea leaves, while about the oxidizing enzyme of Taiwan raw tea leaves Hayasi⁽³⁾ has made a report. While recently very interesting results are reported by Lamb and Roberts.⁽⁴⁾

Now when we make the enzyme solution of tea leaves act on the pure colourless tannin, the tannin becomes reddish brown, like the infusion of black tea. Thus we can infer that the colour of black tea is produced by the change of tannin by enzyme. Moreover, by separating the substance produced by the change of tannin we can ascertain that it is an oxidated product. We have just taken the first step in this study and report the results which have been obtained so far.

THE PROPERTIES OF OXIDIZING ENZYMES.

The tannin and enzyme which we use are as follows.

Tannin Solution: 1% aqueous solution of a crystal of colourless gallo catechin separated from raw leaves⁽⁵⁾.

Enzyme Solution: Grind thoroughly the fresh young leaves in the mortar. Add to it water of twice its quantity and a small quantity of toluol. Make extraction by shaking for two hours. Leave it one night in a cool place and filter by a centrifugal separation. Adding aluminium oxide hydrate, pure, free from alkali ($Al_2(OH)_3$) to the fluid and again by filtering, tannin and other impurities are removed and we get a light yellowish clear solution. This is the enzyme solution. The nature of the oxidizing enzyme contained in this solution may be explained as follows.

A. Peroxidase.

(a) The optimum hydrogen ion concentration.

Put 1 cc., 0.1% guajacol solution, 1 cc., 0.1% hydrogen peroxide, 1 cc Mc Ilvaine's standard buffer solution and 4 cc distilled water in a test tube. Added to it 2 cc enzyme solution and made it act on the solution in the tube at the temperature of 35°C for 15 minutes. And studied the colour-tone of tetraguajacol thus produced, measuring by Rosenheim-Schuster No. 91 tintometer on Lovibond's colour system. As a control experiment, we used enzyme solution boiled for five minutes. The result obtained is as follows.

Table I.

| pH | Red value | pH | Red value | pH | Red value | pH | Red value |
|-----|-----------|-----|-----------|-----|-----------|-----|-----------|
| 3.0 | 7.2 | 3.8 | 15.0 | 4.6 | 15.5 | 5.4 | 13.0 |
| 3.2 | 9.1 | 4.0 | 16.1 | 4.8 | 14.9 | 5.6 | 11.9 |
| 3.4 | 13.1 | 4.2 | 16.3 | 5.0 | 14.2 | 5.8 | 10.9 |
| 3.6 | 14.7 | 4.4 | 16.5 | 5.2 | 14.0 | 6.0 | 10.1 |

So far as this experiment goes the optimum hydrogen ion concentration of peroxidase-action is at pH 4.4.

Moreover we ascertained that within the scope of this experiment, peroxidase action reached its maximum when 1 cc., 0.1% hydrogen peroxide solution was used proportionally with 1~2 cc enzyme solution.

(b) The optimum temperature.

Using 1 cc enzyme solution and 2 cc buffer solution (pH 4.4) and in accordance with the above mentioned experiment (a), measured the optimum temperature of peroxidase action.

Table II.

| Temperature | 25°C | 30°C | 35°C | 40°C | 45°C | 50°C |
|-------------|------|------|------|------|------|------|
| Red value | 6.9 | 7.8 | 9.1 | 10.8 | 10.8 | 11.2 |
| Temperature | 55°C | 60°C | 65°C | 70°C | 75°C | 80°C |
| Red value | 11.3 | 10.7 | 9.3 | 4.9 | 0.9 | 0.1 |

The optimum temperature is 50~55°C.

It is shown by another experiment that peroxidase action is hindered by tannin. In case of measuring peroxidase action, if the quantity of tannin contained in the whole quantity of 10 cc was less than 0.05 mg., there was no hindrance in the colouring reaction and peroxidase could not be adsorbed by aluminium oxide hydrate, and so peroxidase action can be perceived when tannin is removed from enzyme solution containing tannin. As for the causes of these, we must look for their explanation after future study.

B. Oxidase.

(a) The optimum hydrogen ion concentration.

Measured the optimum hydrogen ion concentration in oxidase action by indophenol reaction. Put 1 cc indophenol reagent (the mixture of 1 cc 1% *p*-phenylenediamin solution, 1 cc 1% α -naphthol solution dissolved in 50% alcohol, 2 cc 90% alcohol, 6 cc distilled water. Each must be mixed just before using.), 2 cc buffer solution, 6 cc distilled water, 1 cc enzyme solution in a test tube and mixed them. Then measured the reddish purple colour which was produced after thirty minutes at the temperature of 35°C. As a control test, used enzyme solution boiled for 5 minutes.

Table III.

| pH(*) | Red value | pH(*) | Red value | pH(**) | Red value |
|-------|-----------|-------|-----------|--------|-----------|
| 3.0 | 0.3 | 7.2 | 8.5 | 8.0 | 10.2 |
| 4.0 | 0.8 | 7.4 | 8.8 | 8.3 | 10.0 |
| 5.0 | 2.0 | 7.6 | 9.2 | 8.5 | 9.7 |
| 6.0 | 5.0 | 7.8 | 9.8 | 8.7 | 9.2 |
| 7.0 | 8.0 | 8.0 | 10.2 | | |

(*) McIlvaine standard buffer.

(**) Sorensen's borate buffer

In this study, the optimum hydrogen ion concentration in oxidase action was near pH 8.0.

(b) The optimum temperature.

Using 1 cc enzyme solution and 2 cc buffer solution (pH 8.0), measured the optimum temperature in oxidase action under the above mentioned condition (a).

Table IV.

| Temperature | 25°C | 30°C | 35°C | 40°C | 45°C | 50°C | 55°C |
|-------------|------|------|------|------|------|------|------|
| Red value | 7.8 | 8.7 | 9.8 | 11.0 | 11.6 | 12.6 | 12.2 |
| Temperature | 60°C | 65°C | 70°C | 75°C | 80°C | 85°C | |
| Red value | 12.2 | 10.9 | 8.7 | 5.0 | 3.8 | 2.2 | |

The optimum temperature is 50~65°C.

Oxidase action was not affected by tannin at all and oxidase was not adsorbed to an aluminium oxide hydrate.

THE CHANGE OF CATECHINES EFFECTED BY ENZYME SOLUTION.

We put 2 cc tannin solution, 2 cc enzyme solution, and 1 cc McIlvaine standard buffer solution in a Thunberg tube. After the air in the tube was replaced by oxygen or nitrogen we mixed these solutions. When we had kept the mixture for 20 hours at the temperatures of 35°C, we observed the change of colour. Under the influence of oxygen, the solution gradually became yellowish red or reddish brown. However, under the influence of nitrogen the colour of the solution scarcely changed. On the contrary, the enzyme solution when it was boiled, produced almost no change in nuance in every case. We measured the colour-tones of the solutions which thus changed their colours, by using the tintometer of Rosenheim-Schuster No. 91 on the Lovibond colour system. The result was as follows.

Table V.

| pH | Enzyme solution | Gas in the reaction tube | Degree of colouration | |
|----|--------------------|--------------------------|-----------------------|------|
| | | | Yellow | Red |
| 5 | boiling boiling | Oxygen | 4.1 | 19.1 |
| | | Nitrogen | 1.0 | 4.0 |
| | | Oxygen | 1.1 | 3.9 |
| | | Nitrogen | 0.8 | 1.0 |
| 6 | boiling boiling | Oxygen | 8.0 | 29.0 |
| | | Nitrogen | 1.8 | 5.0 |
| | | Oxygen | 1.8 | 8.0 |
| | | Nitrogen | 0.7 | 1.3 |
| 7 | boiling boiling | Oxygen | 23.3 | 19.5 |
| | | Nitrogen | 8.0 | 14.2 |
| | | Oxygen | 9.1 | 16.0 |
| | | Nitrogen | 2.3 | 2.5 |

The degree of the change of colour differs according to pH and the colour becomes more intense and the colour-tone differs as pH nears neutrality. In acid case, under the influence of nitrogen gas, there is scarcely any change of colour, and in the case of boiled enzyme solutions, under the influence of oxygen, there is almost no change of colour, either. In the case of pH there is more or less colouring in every case, but compared with the case where the enzyme is made to act, the degree of colouring is very slight.

Still, for the sake of comparison, we observed the reaction in the same way, using *d*-catechin, tea-tannin and synthetic bisflavpinacol⁽³⁾.

Although we cannot see what kind of chemical change happened, it is clear

Table VI.

| pH | Gas in the reaction tube | Tannin solution | Enzyme solution | Degree of colouration | |
|----|--------------------------|--------------------|-----------------|-----------------------|------|
| | | | | Yellow | Red |
| 6 | Oxygen | <i>d</i> -catechin | | 10.2 | 30.0 |
| | | | boiling | 1.0 | 4.1 |
| | | tea tannin | | 14.8 | 37.3 |
| | | | boiling | 2.6 | 4.5 |
| | | bisflavpinacol | | 22.2 | 38.3 |
| | | | boiling | 3.0 | 6.3 |

from the result above that the enzyme solution which we use here, exerts action on not only catechin but also on tannin and has the action to change them into reddish brown substances.

Accordingly we know that tannin is changed by the enzyme action and is coloured as the result, and also that oxygen is necessary in this case. In the process of manufacturing black tea, such a change happens naturally and we can say that the colour of black tea is thus produced.

OXIDATED PRODUCT OF CATECHINS EFFECTED BY THE ENZYME.

In order to get the coloured-substance through the above experiment, we made an experiment under the proper condition and we got the precipitation of coloured substance from the same reaction in both cases of *d*-catechin and gallocatechin.

Oxidation of *d*-catechin: dissolved 1 g. *d*-catechin in 30 cc water, and added to it 30 cc enzyme solution and 10 cc buffer solution (pH 6.0). Letting in oxygen, stirred it. Left it for 24 hours at the temperature of 40°. Then the mixed solution gradually changed from yellowish red to red. And finally yellowish red precipitation was produced. Next separated the precipitation by filtration and washed with water and dried it. And then we got reddish brown powder which was insoluble in water and was difficult to dissolve in alcohol.

Oxidation of gallocatechin: dissolved 2 cc gallocatechin in 20 cc water. Added 50 cc enzyme solution and 10 cc buffer solution (pH 6.0), and let in oxygen, and made it act on the mixed solution for about 60 hours at the temperature of 40°. And then the solution gradually changed from yellowish red to red and finally reddish brown precipitation was produced. Separated the precipitates by filtration and washed with water and dried it. Then the reddish brown powder, which was insoluble in water and difficult to dissolve in alcohol, was produced.

The elementary composition of these substances is as follows.

| | C(%) | H(%) | O(%) |
|--|-------------|-----------|-----------------|
| <i>d</i> -catechin $C_{15}H_{14}O_7$ | 62.07 | 4.83 | 33.10 |
| Oxidated product of <i>d</i> -catechin | 50.07~51.44 | 5.04~5.30 | 44.07 (average) |
| Gallocatechin $C_{18}H_{14}O_7$ | 57.81 | 4.61 | 37.58 |
| Oxidated product of gallocatechin | 50.11~51.78 | 4.76~4.90 | 44.22 (average) |

The coloured substance produced by enzyme action shows a remarkable decrease of carbon and a remarkable increase of oxygen. Therefore, we consider the change as a kind of oxidation.

We get the reddish brown acetyl compounds by acetylating the oxidated products above mentioned with pyridine and anhydrous acetic acid in both cases. If we compare the acetyl compound of catechin with the acetyl compound of oxidated product from catechin, as can be seen by the following chart, there is a little quantity of acetyl radicals in the latter and then a decrease of free hydroxyl radicals. And this fact is a notable change

| | CH ₃ O(%) |
|--|----------------------|
| Acetyl compound of <i>d</i> catechin C ₁₅ H ₁₀ O(O CO CH ₃) ₅ | 43 00 |
| Acetyl compound of oxidated <i>d</i> catechin | 15 06~15 17 |
| Acetyl compound of gallo catechin C ₁₅ H ₁₀ O(O CO·CH ₃) ₅ | 46 23 |
| Acetyl compound of oxidated gallo catechin | 36 34~36 45 |

When tannin is oxidized by enzyme, probably after various steps, it becomes an insoluble substance, that is to say, phlobaphene. In the course of the steps a soluble oxidized product is obtained, and that is how the colour of black tea is produced.

- (1) Deuss Rec Trav chun, **42**, 496, (1923)
 Shaw Thea Tannin (1932)
 Tujimura Sci Paper Institute Physical and Chem Research **14**, 261, (1930), **26**, 186, (1935)
 - (2) Osima J Agr Chem Soc Japan, **12**, 1, (1936)
 - (3) Hayasi J Soc Trop Agr Taiwan, Japan, **6**, 388, (1934)
 - (4) Lamb and Roberts Nature **18**, 867, (1939)
-

ABSTRACTS

from

TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noted.)

Researches on Bamboos in Taiwan as a Raw Material for Pulp. Part III.

(pp. 621~625)

By Minoru TUTIYA and Setuo FUKUHARA.

(Industrial Research Institute of Taiyu, Taiwan, Received June 4, 1940)

Studies on the Vegetable Tannins in Taiwan. Part 6.

Manufacture of Tanning Extract from the Bark
of *Acacia confusa*. II.

(pp. 626~630)

By Yasuyosi OSIMA, Zensaburo SIRAKI and Zenyu HYO.

(Agricultural Chemical Department, Taihoku Imperial University, Taiwan;

Received June 8, 1940)

As the result of studying the extraction of bark with alkali or acid solution, we found that the maximum yield of tannin could be obtained with 0.1% HCl, H₂SO₄ or SO₂ solution. We made tannin extract with 0.1% SO₂ solution, which we thought most practical, and examining the chemical properties, diffusion velocity into gelatin-gel, and absorption amount of tannin by hide powder, we got good results in all cases.

Chemical Researches on the Pulp Woods of Manchoukuo. Part VI.

Fibre-length, Chemical Analysis and Cooking Experiment
of the Hard Wood.

(pp. 631~640)

By Masuzo SHIKATA and Yoshitsugu KIMURA.

(Kyoto Imperial University; Received June 10, 1940.)

In this paper, the researches on the chemical components, fibre-length, and cooking experiments of hard woods are given.

The species of the woods employed are as follows ;

| Japanese name | Scientific name | Annual rings |
|---------------|-------------------------------|--------------|
| Ominonire | <i>Ulmus Macrocarpa</i> Hance | 107 |
| Ohyonire | <i>Ulmus Laemata</i> , Mayr | 71 |

1. Physical properties.

The distribution of fibre-lengths is as shown in Table 1.

Table 1. The Distributions of Fibre-length (Interval 0.1 mm).

| length (mm) | Ominonire | Ohyonire | Length (mm) | Ominonire | Ohyonire |
|-------------|-----------|----------|-------------|-----------|----------|
| 0.3~0.4 | 4.0 | 0.6 | 1.5~1.6 | 0.4 | 3.6 |
| 0.4~0.5 | 5.4 | 1.6 | 1.6~1.7 | 0 | 3.6 |
| 0.5~0.6 | 5.8 | 2.6 | 1.7~1.8 | 0 | 2.8 |
| 0.6~0.7 | 8.0 | 3.0 | 1.8~1.9 | 0.2 | 0.4 |
| 0.7~0.8 | 8.8 | 3.4 | 1.9~2.0 | 0 | 0.8 |
| 0.8~0.9 | 19.2 | 5.2 | 2.0~2.1 | 0 | 0.4 |
| 0.9~1.0 | 15.6 | 9.6 | 2.1~2.2 | 0 | 0.6 |
| 1.0~1.1 | 15.4 | 9.2 | 2.2~2.3 | 0 | 0 |
| 1.1~1.2 | 11.2 | 18.0 | 2.3~2.4 | 0 | 0 |
| 1.2~1.3 | 7.2 | 15.2 | 2.4~2.5 | 0 | 0 |
| 1.3~1.4 | 2.4 | 13.0 | 2.5~2.6 | 0 | 0.4 |
| 1.4~1.5 | 1.6 | 6.0 | | | |

The data given in Table 1 are graphically shown in Figure 1. Those data

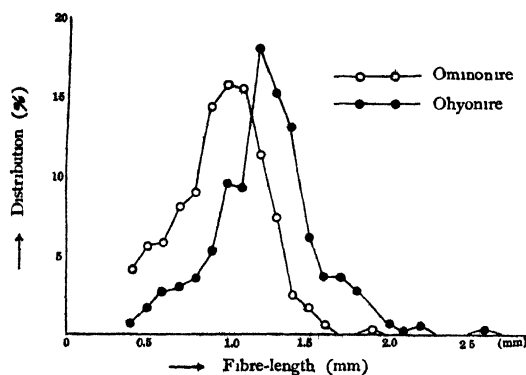


Fig. 1.

show that the fibre-lengths of these hard woods are not so long as those of soft woods, but are nearly equal to those of other hard woods.

2. Chemical analysis of wood components.

The results of analysis of the chemical components of these woods are given in Table 2.

Table 2. Chemical Components (% Oven dry).

| Components | Species | Ominonire | Ohyonire | Components | Species | Ominonire | Ohyonire |
|-------------------------|---------|-----------|----------|------------------------------|--------------------|-----------|----------|
| Alcohol-benzene-soluble | | 1 97 | 1 48 | Hemi-cellulose | | 22 01 | 23 10 |
| Water soluble | | 0 87 | 1 12 | Nitrogen | | 0 12 | 0 15 |
| Hot-water-soluble | | 1 59 | 1 65 | Crude protein | | 0 76 | 0 95 |
| 1% NaOH-soluble | | 13 16 | 16 76 | Ash | | 1 03 | 0 83 |
| Crude cellulose | | 54 85 | 56 22 | Methoxyl | | 6 92 | 7 30 |
| α -cellulose | | 36 61 | 39 96 | Methoxyl/Lignin $\times 100$ | | 30 70 | 32 42 |
| β -cellulose | | 9 22 | 10 21 | Ca-pectic acid | | 2 31 | 2 77 |
| γ -cellulose | | 9 02 | 6 05 | | | | |
| Lignin | | 22 54 | 22 52 | In Total cellulose | α cellulose | 66 75 | 71 08 |
| Pentosan | | 21 90 | 23 01 | | β cellulose | 16 81 | 18 16 |
| Mannan | | 0 00 | 0 00 | | γ cellulose | 16 44 | 10 76 |
| Galactan | | 0 11 | 0 09 | Volume weight | | 0 49 | 0 55 |

3. Cooking experiments.

The cooking experiments with the Ca-sulphite process and sulphate process were carried out under the conditions given in Table 3.

Table 3. The Conditions of Cooking Experiments.

| Run Number | A-1 | A-2 | A-3 | B-1 | B-2 | C-1 |
|---------------------------|------------------------------|---------------|-------|---------|------|--|
| Method | Ca-Sulphite Process | | | | | Craft Process |
| Condition | | | | | | |
| Cooking solution | CaO 1 (%) | " | " | " | " | NaOH 7 5 (%) |
| | Total SO ₂ 7 (%) | " | " | " | " | Na ₂ S 1 75 (%) |
| | Free SO ₂ 5 8 (%) | " | " | " | " | Na ₂ CO ₃ 1 75 (%) |
| Chip/ cooking solution | 20 g/100 cc | 14 3 g/100 cc | " | " | " | " |
| Penetration | 110°C, 4 at p * | " | " | " | " | " 2 at p |
| | 2 hrs | " | " | " | " | " |
| Main cooking | 130°C, 6 at, p ** | " | " | 135°C " | " | 160°C " |
| | 3 hrs | 5 | 7 | 5 | 7 | 2 |
| Total cooking time | 8.6 hrs | 10.25 | 12 75 | 11 0 | 13 0 | 7 25 |

* at p Atmospheric pressure.

** 6 at p Maintain 6 at p by blowing.

The chemical components of unbleached pulps are given in Table 4.

Table 4. Cooking Data.
The Analysis of Unbleached Pulps and Yield.

| Wood | Components | Run Number | A-1 | A-2 | A-3 | B-1 | B-2 | C-1 |
|-----------|-------------------------------------|------------|-------|-------|-------|-------|-------|--------|
| Ominonire | Ash | | | 0.59 | 0.79 | 0.92 | 0.65 | 2.29 |
| | α -cellulose | | | 80.42 | 83.58 | 78.88 | 84.83 | 28.26 |
| | β -cellulose | | | 7.33 | 6.81 | 11.95 | 5.66 | 8.62 |
| | Pentosan | | | 5.01 | 3.87 | 5.82 | 4.59 | 13.24 |
| | Copper number | | | 3.82 | 3.20 | 2.72 | 2.62 | 0.62 |
| | Roe's number | | | 6.07 | 4.82 | 4.51 | 3.16 | 3.02 |
| | Yield to chip | | | 45.84 | 47.37 | 39.37 | 37.5 | 38.65 |
| | Yield to 1 m ³ wood (kg) | | | 225 | 232 | 192 | 184 | 189 |
| Ohyonire | Ash | | 2.11 | 0.55 | 0.40 | 0.72 | 0.62 | 2.56 |
| | α -cellulose | | 71.91 | 82.26 | 84.71 | 82.45 | 84.69 | 85.54 |
| | β -cellulose | | 12.48 | 7.71 | 6.95 | 10.48 | 4.64 | 10.09* |
| | Pentosan | | 7.26 | 5.01 | 2.77 | 4.93 | 6.17 | 10.35 |
| | Copper number | | 2.57 | 2.26 | 2.97 | 2.60 | 2.65 | 0.76 |
| | Roe's number | | 12.79 | 4.64 | 3.03 | 3.04 | 2.86 | 2.17 |
| | Yield to chip | | 52.47 | 46.35 | 47.89 | 42.75 | 41.70 | 39.54 |
| | Yield to 1 m ³ wood (kg) | | 289 | 255 | 263 | 235 | 229 | 217 |

4. Bleached pulps.

The unbleached sulphite- and sulphate-pulps of each wood were bleached by the two stages method.

After applying chlorine gas amounting to about 75% of theoretical bleach requirement, which was calculated by "Roe-number," they were washed with about 0.03% NaOH and water, and steeped in bleaching powder solution of about 0.18% for 0.5 hours to 7 hours at room-temperature.

The chemical components of each bleached pulp are shown in Table 5.

Table 5. Bleached Pulps.
The Analysis of Bleached Pulps and Yields.

| Wood | Components | Run Number | A-1 | A-2 | A-3 | B-1 | B-2 | C-1 |
|-----------|-------------------------------------|------------|-----|-------|-------|-------|-------|-------|
| Ominonire | Ash | | | 0.28 | 0.31 | 0.28 | 0.28 | 0.44 |
| | α -cellulose | | | 81.84 | 86.79 | 82.01 | 88.84 | 89.05 |
| | β -cellulose | | | 17.01 | 9.49 | 10.70 | 6.50 | 8.87 |
| | γ -cellulose | | | 1.15 | 3.62 | 7.29 | 4.66 | 2.08 |
| | Pentosan | | | 4.32 | 3.41 | 5.00 | 3.75 | 10.35 |
| | Copper number | | | 2.55 | 1.06 | 1.14 | 1.10 | 0.54 |
| | Yield to chip | | | 37.48 | 39.47 | 34.34 | 33.14 | 29.07 |
| | Yield to 1 m ³ wood (kg) | | | 184 | 194 | 168 | 162 | 151 |

| | | | | | | | |
|----------|-------------------------------------|-------|-------|-------|-------|-------|-------|
| Ohyonire | Ash | 1.00 | 0.33 | 0.18 | 0.30 | 0.38 | 0.59 |
| | α -cellulose | 82.94 | 84.06 | 88.38 | 87.84 | 87.59 | 89.84 |
| | β -cellulose | 8.54 | 11.86 | 6.19 | 10.54 | 7.16 | 9.61 |
| | γ -cellulose | 8.52 | 4.08 | 5.42 | 1.62 | 4.25 | 0.55 |
| | Pentosan | 6.13 | 4.43 | 2.68 | 4.08 | 2.38 | 9.07 |
| | Copper number | 4.47 | 0.95 | 1.41 | 0.65 | 0.73 | 0.61 |
| | Yield to chip | 38.41 | 39.89 | 42.11 | 37.57 | 36.64 | 34.73 |
| | Yield to 1 m ³ wood (kg) | 211 | 218 | 231 | 207 | 202 | 191 |

Conclusion.

The sulphite pulps showed lower pentosan content and lower ash content.

In general, Ohyonire were superior to Ominonire with regard to the pulp-yields and to the α -cellulose contents, pentosan.

It seemed that with respect to the sulphite process, low temperature and long time cooking is comparatively good.

Biochemical Studies on Glutathione. Report XIII.

Relation between the Administration of Diet and the Glutathione
Content of Arterial and Venous Bloods.

(pp. 641~648)

By Masayoshi OGAWA.

(Department of Nutrition, College of Medicine, Nippon University,

Received June 3, 1940)

In the previous communication the author reported some correlations between the glutathione contents of arterial and venous blood of normal rabbit (glutathione was determined by the method of Okuda and Ogawa.).

In the present report he investigated the effect of administration of diet upon the glutathione (GSH, GS-SG) content of arterial and of venous bloods, employing several normal rabbits.

These animals were phlebotomized at intervals before and after feeding. The results obtained are shown in the following table.

Glutathione Content of Blood before and after Meal Feeding.

| Hours observed after meal. | GSH | | GS-SG | | Total | |
|-------------------------------|----------------|---------------|------------------|----------------|------------------|--------------|
| | Arterial | Venous | Arterial | Venous | Arterial | Venous |
| before meal | 100 (90.1) | 100 (100) | 100 (132.3) | 100 (100) | 100 (100.5) | 100 (100) |
| 1/2 hours after meal | 93.0 (88.2) | 97.0 (100) | 167.9 (189.9) | 104.5 (100) | 123.1 (124.8) | 100 (100) |

| | | | | | | |
|------------------------|-----------------|----------------|------------------|----------------|------------------|----------------|
| 1 hours after meal | 97.0 (89.1) | 98.3 (100) | 145.3 (169.6) | 105.9 (100) | 114.9 (114.5) | 100.5 (100) |
| 2 hours after meal | 102.3 (93.1) | 101.1 (100) | 150.7 (164.9) | 108.0 (100) | 121.7 (118.8) | 103.9 (100) |
| 4 hours after meal | 103.1 (81.4) | 104.9 (100) | 143.9 (190.2) | 113.3 (100) | 119.0 (111.2) | 107.1 (100) |
| 6 hours after meal | 99.4 (93.4) | 95.8 (100) | 145.4 (171.4) | 108.0 (100) | 115.0 (116.1) | 99.1 (100) |
| 8 hours after meal | 93.8 (86.1) | 104.3 (100) | 151.5 (172.7) | 97.5 (100) | 111.4 (108.7) | 102.4 (100) |
| 10 hours after meal | 100.3 (87.1) | 100.6 (100) | 107.3 (145.3) | 92.6 (100) | 103.6 (105.2) | 98.0 (100) |
| 12 hours after meal | 101.7 (89.3) | 105.1 (100) | 98.5 (132.8) | 87.8 (100) | 100.4 (101.4) | 99.6 (100) |

As shown in the above table, in every case, the GSH contents of arterial blood is less than that of venous blood, while the GS-SG contents of arterial blood is greater than that of venous blood. The total glutathione contents are the same in the case of fasting.

It is a very interesting fact that GS-SG content of arterial blood is conspicuously increased immediately after feeding and within 10~12 hours it returns to normal condition, while GSH and GS-SG content of venous blood are not conspicuously influenced throughout the experiment.

Studies on Ascorbic Acid (III).

On the Action of Ascorbic Acid on Glutathione. (I).

(pp. 649~652)

By Kichinosuke FUJIMURA.

(The Institute of Chemical Research, Kyoto Imperial University;

Received June 12, 1940.)

Exchangeable Calcium and Magnesium of Soils in Tyōsen. (I.)

(pp. 653~662)

By Mitsu-Hideo.

(Agricultural Experiment Station, Government General of Tyōsen;

Received June 6, 1940.)

On the Metabolism of Organic Acids by Bacteria. I~II.

(pp. 663~686)

By S. TADA.

(Agricultural Chemical Laboratory, Tokyo Imperial University ;

Received June 12, 1940)

Studies on the Value of Chemicals as Manure for *Juncus effusus* L. var. *decipiens* BUCH.

Report II.

On the Effect of Different Kinds of Potash Salts.

(pp. 687~695)

By H. SUTOH.

(The Prefectural School of Agriculture and Forestry, Masuda, Simane, Japan ;

Received June 8, 1940.)

A report has already been made by the author on the relative value of different kinds of nitrogen compounds. In this paper the result of experiments carried on with different kinds of potash salts will be presented. The potassium salts used in the present experiment were potassium carbonate, potassium sulphate, potassium chloride, potassium bromide, potassium iodide, potassium bichromate, potassium chlorate and potassium permanganate as potassium source. Several lots of these salts, of no potassium salt, and of no manure, were set up in a greenhouse. The conditions of growth, yield, together with the quality of the rush were investigated.

The results obtained are summarized as follows :

(1) The rush in the lots of K_2CO_3 , K_2SO_4 , KCl, KBr and $KMnO_4$ were all observed to cause the normal growth in length of the stem till late in October and then slackened. In winter season, the top of the rush began to wilt and the wilting gradually extended down toward the root as far as 2/5 part of the stem. This wilting was especially remarkable in the lots of KBr and $KMnO_4$.

(2) The tillering continued as late as the harvesting time in the lots of K_2SO_4 , $KMnO_4$, KCl, K_2CO_3 , and also those with no potassium, tillers increasing in number ranging from about 18 in no potassium lots to about 26 times the original number in the K_2SO_4 -lots.

(3) The comparative yield of air-dried stems was 135 in the lot of K_2SO_4 , 121 in the KCl-lot, 119 in the K_2CO_3 lot, 117 in the $KMnO_4$ -lot, 114 in the KBr-lot, and 100 in no potassium lot.

(4) Under the condition in which this experiment was carried out KCl was useful in increasing the yield of long stem, although the total yield in this case was less than that in K_2SO_4 lot, as against the supposition that it would be specially beneficial for the rush on account of its possession of Cl. What the writer

found out is that KCl is superior to K_2SO_4 in producing the rush of better quality, as the soil culture also proved.

(5) When KMnO_4 was used as the potassium source as well as a stimulant in so large a quantity as $25 \text{ kg/tan}(\text{K}_2\text{O})$, its beneficial effect on the crop could hardly be noticed. A considerable large number of tillers were, however, produced in the lots.

(6) Though $\text{K}_2\text{Cr}_2\text{O}_7$ is an oxidizing agent chemically resembling KMnO_4 , it was found very harmful, making the crops wither in a short period. The negative ion ($\text{Cr}_2\text{O}_7^{--}$) may be harmful when a valency of Cr is as high as 6. This crop in soil culture, however, did not suffer so much from the action of this salt as in sand culture. Further investigation will be necessary to determine its useful concentration.

(7) In the present experiment KBr and KI gave some interesting results; KBr -lot gave an yield of 94% as high as that of KCl -lot, but gave so bad a result that the plants all died. Further investigation is necessary as to the quantity of these salts useful as a stimulant.

(8) KClO_3 was harmful, but it was not so harmful as $\text{K}_2\text{Cr}_2\text{O}_7$ or KI . The harm it did to the rush was noted to be slack in appearing in comparison with that of $\text{K}_2\text{Cr}_2\text{O}_7$ or KI , and that some plants could withstand complete withering although the stems were slender and weak.

(9) The author's attention has also been directed toward the relative position on the periodic chart of the atoms of which negative parts of compounds are composed.

Bulletin of the Agricultural Chemical Society of Japan.

ABSTRACTS

from

TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noted.)

Studies on the Lactic Acid Bacteria Isolated from Mashs of Various Kinds of Cereals.

(pp. 697~714)

By KAKUO KITAHARA.

(Department of Agriculture, Kyoto Imperial University, Received July 3, 1940.)

From various kinds of mashs, 42 strains of lactic acid formers were isolated at different temperatures of 30~52°. All the strains were found to be Gram-negative, non-motile, non-spore-bearing and produced lactic acid from glucose and fructose.

According to their morphological and physiological characteristics mentioned in the previous paper (This Journal **14** (1938), 1449), the classification of these bacteria was proposed as follows:

- Group I. *Streptococcus* (*Enterococcus*) (2 strains);
Sc. faecalis (1), *Sc. glycerinaceus* (1)
- Group II. *Pedococcus* (6 strains);
Pc. hennebergi (2), *Pc. lindneri* (4)
- Group III. *Leuconostoc* (2 strains);
Leuc. mesenteroides α -type (1), *Leuc. mesenteroides* β -type (1)
- Group IV. *True-Lactobacillus* (Homo-fermenters, without catalase) (8 strains);
L. delbrückii (2), *L. acidophilus* (1), *L. casei* (1), *L. plantarum* (1), *L. xylosum* nov. sp. (3)
- Group V. *Beta-Lactobacillus* (Hetero-fermenters, without catalase) (15 strains);
L. brevis α -type (1), *L. fermentum* α -type (7), *L. betadelbrückii* nov. sp. (1), *L. brevis* β -type (3), *L. fermentum* β -type (3)
- Group VI. *Wild-Lactobacillus* (revealed catalase action) (11 strains);
L. thermophilus (6), *L. ciliatus* nov. sp. (2), *L. caneus* nov. sp. (3).

The very characteristic natures of the newly recorded species *L. xylosum*, *L. betadobacilli*, *L. ciliatus* and *L. canus* were pointed out.

Comparing with the distribution of the strains of lactic acid bacteria between the dairy products and the mashes, it was concluded that *Streptococci* and *True-Lactobacilli* were abundant in dairy products. *Pediococci*, which were never isolated with dairy products, were found to be abundantly present in the mashes. The distributions of *Tetracoca* and of *Escherichia* were limited to the dairy products. *Beta-Lactobacilli* and *Wild-Lactobacilli* were more frequently isolated with the mashes, while *Leuconostoc* was distributed equally between the mashes and the dairy products.

Exchangeable Calcium and Magnesium of Soils in Työsen.

(pp. 715~724)

By MISU-Hideo.

(Agricultural Experiment Station, Government General of Työsen,
Received June 6 1940)

Über die Fabrikation des Alkohol aus Überrest des Maniokmehls. (Amyloverfahren)

(SS. 725~730)

Von Masao TAKESITA.

(The Institute of Research on Chemical Industry, Government General of
Taiwan, Japan, Eingegangen am 24 6 1940)

Studies of Fiber from *Cannabis sativa* (I)

Relation of Constituents of Fiber to the Growth Periods and
Sex. On the Value of the Fiber from Wooden
Parts of Hemp as Pulp.

(pp. 731~738)

By Yoshijirō KIHARA, Hikonojō NAKAHARA, and Gorō KODERA.

(Agri. Chem. Laboratory, Tokyo Imp. Univ., Received June 29, 1940)

The wooden part of the hemp has with its growth a tendency to decrease in quantity of ash, alcohol-benzene extract and the total soluble sugar, while the crude protein and the total cellulose are increased, and lignin, pentosan and α -cellulose remain unchanged.

With respect to the sex of this plant, in staminate, alcohol-benzene extract, crude protein, total soluble sugar and pentosan can be found in higher proportion than in pistillate, but the total cellulose as well as α -cellulose in pistillate, appear in a larger percentage than in staminate. The analytical quantity of ash and lignin is the same in both sexes.

The constituents of the fiber, which is made from the wooden part of hemp, are similar to those of bagasse, "Onigaya" (*Miscanthus sinensis*) in Taiwan, and kanaff and the fiber length is similar to that of bagasse. Its length (0.5~1.5 mm long) is likewise rather too short to be used as pulp, without mixing with other wood pulp.

Chemische Bestandteile des Tees. I. Mitteilung.

Arginin aus Giokuro.*

(SS. 739~740)

Von Yaziro SAKAFO.

(Aus d. Institut für Tee Kyo'o, Eingegangen am 4. 7. 1940.)

Neulich hat der Verfasser *Arginin* in Form von Pikrolonat (F. 231) in dem von Giokuro hergestellten Wasserextrakt festgestellt. Die Ausbeute an Pikrolonat aus 500 g Tee betrug 0.84 g.

Giokuro ist eine der besten japanischen grünen Teesorten.

Chemische Untersuchung über die Bestandteile der Roskastaniensamen. III. Mitteil.

(SS. 741~750)

Von M. KANDATSU.

(Agrikulturchem. Institut d. Univers. Tokyo; Eingegangen am 1. 7. 1940.)

Determination of the Total Vitamin B₁ in Natural Substances.

(pp. 751~754)

By Yosito SAKURAI and Tyoten INAGAKI.

(Research Laboratory of Meiji Sugar Co.; Received July 1, 1940.)

The authors used the mixture of takadiastase and "Sumeretin," a ¹Japanese preparation, to convert the combined forms of vitamin B₁ into free state. The digestion period was over 24 hours at 37~40°C and pH 4~5.

Researches on Bamboo in Taiwan as a Raw Material for Pulp. Part IV.

On the Ashes and Some Characters of Pulps and " α -Celluloses"
Obtained by Different Digesting Methods.

(pp. 755~760)

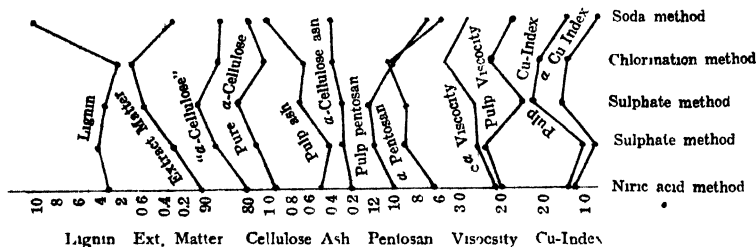
By MINORU TUIYA and Masao IMAI.

(Industrial Research Institute of Taiwan, Received July 4, 1940)

As a sample we used the stem, part of under branches, of 3 year old Keitiku. The methods used were as mentioned bellow, nitric acid method, caustic soda method, sulphate method, chlorination method and sulphite method (this composition is $Mg(HSO_3)_2$ and a little quantity of $MgSO_3$ in the state of solution).

The quantities and components of ashes were determined as shown in Table I and II.

The Fig. and Table III. represent the differences of quantities and values of pulps and " α -celluloses" which were obtained by each digesting method. The arrangement is made in the order of the quantities of α -cellulose ashes.



We may summarize the results as follows:—

1. Since 70% of ashes of Keitiku is composed of natrium and potassium, to obtain a little quantity of ashes the acid digesting method is better than the alkali.
2. Alkali method excudes more silica than the acid method.
3. The least quantity of ash is obtained by the nitric acid method which is the combined method of acid and alkali.
4. The pulps treated with 17.5% NaOH lower the quantities of pentosan and Cu-index, elevate the viscosity and diminish ashes to about half.
5. For the extinguishment of pentosan the alkali method is better than the acid one.
6. As the digesting methods for bamboo, each method has merits and defects, but among those the nitric acid method is the most recommendable and next is the sulphate method, in those adopted methods.

TABLE I.
Quantity of Ashes and Percentage of its Components.

| Kind | Components (%) | Ash | SiO ₂ | Fe ₂ O ₃ | SO ₃ | P ₂ O ₅ | CaO | MgO | MnO | K ₂ O | N ₂ O |
|---------------------|-----------------|------|------------------|--------------------------------|-----------------|-------------------------------|-------|------|------|------------------|------------------|
| Keitku | | | | | | | | | | | |
| | | 1.7 | 12.20 | 4.86 | 1.70 | 1.62 | 1.78 | 0.97 | 1.94 | 12.70 | 56.30 |
| Nitric acid Method | Unbleached pulp | 0.53 | 43.76 | 10.35 | 9.56 | — | 6.92 | 3.62 | 2.50 | 13.38 | 3.02 |
| | Bleached pulp | 0.52 | 67.52 | 12.83 | 1.78 | — | 8.29 | 0.27 | 1.64 | 0.79 | 3.68 |
| | α-Cellulose | 0.21 | 50.92 | 29.57 | 4.92 | — | 1.64 | — | 1.60 | 1.64 | 5.33 |
| Caustic soda Method | Unbleached pulp | 1.29 | 13.81 | 17.23 | 7.95 | — | 8.45 | 4.52 | 9.08 | 4.54 | 31.86 |
| | Bleached pulp | 1.11 | 11.73 | 21.69 | 3.47 | — | 8.47 | 3.91 | 7.72 | 5.60 | 32.50 |
| | α-Cellulose | 0.42 | 14.27 | 24.09 | 3.89 | — | 17.74 | 3.91 | 3.72 | 8.29 | 22.65 |
| Sulphate Method | Unbleached pulp | 0.79 | 19.61 | 23.85 | 6.64 | — | 15.79 | 3.81 | 2.64 | 3.38 | 18.21 |
| | Bleached pulp | 0.43 | 13.57 | 20.71 | 11.10 | — | 27.38 | 7.57 | 2.50 | 6.10 | 2.90 |
| | α-Cellulose | 0.28 | 17.90 | 31.00 | 6.60 | — | 17.81 | 6.51 | 2.32 | 7.58 | 5.08 |
| Chlorination Method | Unbleached pulp | 0.73 | 35.44 | 16.95 | 0.25 | — | 1.72 | 1.23 | 3.39 | 40.58 | 2.47 |
| | Bleached pulp | 0.67 | 42.20 | 16.75 | 0.27 | — | 1.06 | 1.33 | 3.74 | 29.05 | 1.03 |
| | α-Cellulose | 0.41 | 52.28 | 3.71 | 0.14 | — | 1.28 | 2.45 | 6.72 | 27.20 | 0.86 |
| Sulphite Method | Unbleached pulp | 0.72 | 54.74 | 24.34 | 1.90 | — | 1.72 | 5.70 | 7.38 | 0.27 | 2.93 |
| | Bleached pulp | 0.71 | 59.26 | 18.78 | 1.64 | — | 1.67 | 6.73 | 5.99 | 1.47 | 3.13 |
| | α-Cellulose | 0.33 | 63.65 | 18.64 | 0.88 | — | 1.01 | 8.12 | 6.25 | 0.15 | 0.45 |

TABLE II.
mg of the Components to 100 mg of Bamboo and Percentage.

| Kind | Components (%) | Ash | SiO ₂ | Fe ₂ O ₃ | SO ₃ | CaO | MgO | MnO | P ₂ O ₅ | K ₂ O | N ₂ O |
|------|----------------|-----|------------------|--------------------------------|-----------------|-----|-----|-----|-------------------------------|------------------|------------------|
|------|----------------|-----|------------------|--------------------------------|-----------------|-----|-----|-----|-------------------------------|------------------|------------------|

| Kantika { mg % | | 1770.00 100.00 | 215.94 100.00 | 86.02 100.00 | 30.09 100.00 | 31.50 100.00 | 17.16 100.00 | 34.33 100.00 | 28.67 100.00 | 224.79 100.00 | 996.51 100.00 |
|------------------------|---------------------|-------------------|----------------------|----------------------|---------------------|---------------------|---------------------|---------------------|-------------------|----------------------|----------------------|
| Nitric acid Method | Unbleached pulp | { mg % | { 191.00 107.8 | { 118.16 54.70 | { 22.90 26.00 | { 18.25 60.66 | { 14.50 45.90 | { 3.11 13.80 | { — — | { 25.54 11.36 | { 5.75 0.57 |
| | Bleached pulp | { mg % | { 175.00 9.80 | { 83.61 38.71 | { 19.76 22.90 | { 3.11 10.30 | { 13.21 41.80 | { 0.47 2.70 | { 2.87 8.30 | { 6.44 6.13 | { 0.64 3.03 |
| | α -Cellulose | { mg % | { 57.00 3.00 | { 50.92 29.02 | { 16.85 19.50 | { 2.80 9.30 | { 0.93 2.90 | { 0.93 2.70 | { — — | { 0.93 4.13 | { 3.03 0.30 |
| Caustic soda Method | Unbleached pulp | { mg % | { 377.00 21.20 | { 51.09 23.60 | { 63.75 7.40 | { 29.41 9.70 | { 31.26 99.09 | { 16.72 97.40 | { — — | { 16.79 7.46 | { 121.88 13.20 |
| | Bleached pulp | { mg % | { 293.00 16.50 | { 34.01 15.70 | { 61.16 7.41 | { 10.06 3.34 | { 24.56 77.80 | { 11.36 66.10 | { — — | { 16.24 7.22 | { 94.25 9.40 |
| | α -Cellulose | { mg % | { 98.00 5.50 | { 1.39 0.60 | { 2.36 2.70 | { 0.38 1.26 | { 1.73 54.80 | { 0.38 2.20 | { — — | { 0.81 3.60 | { 2.21 0.20 |
| Sulphate Method | Unbleached pulp | { mg % | { 249.00 14.00 | { 47.06 21.70 | { 57.44 66.70 | { 15.93 52.90 | { 30.71 97.30 | { 9.14 53.20 | { — — | { 7.11 3.16 | { 43.69 4.38 |
| | Bleached pulp | { mg % | { 116.00 6.50 | { 14.91 6.90 | { 22.78 26.40 | { 21.21 40.50 | { 30.11 95.40 | { 8.32 48.40 | { — — | { 6.70 2.90 | { 3.19 0.30 |
| | α -Cellulose | { mg % | { 67.00 3.70 | { 11.90 5.50 | { 20.77 24.10 | { 5.08 16.80 | { 11.93 37.80 | { 4.36 25.40 | { — — | { 5.08 2.20 | { 3.04 0.30 |
| Chlorination Method | Unbleached pulp | { mg % | { 330.00 18.60 | { 116.95 54.10 | { 45.57 52.90 | { 0.82 2.70 | { 5.67 17.90 | { 11.18 34.30 | { — — | { 133.91 59.50 | { 8.15 9.91 |
| | Bleached pulp | { mg % | { 271.00 15.20 | { 113.94 52.80 | { 45.22 52.50 | { 0.72 2.30 | { 2.86 9.06 | { 3.59 20.90 | { — — | { 78.43 34.80 | { 2.76 0.20 |
| | α -Cellulose | { mg % | { 145.00 8.10 | { 75.80 35.00 | { 12.62 14.60 | { 0.20 0.60 | { 1.85 5.86 | { 3.55 20.60 | { — — | { 39.44 17.50 | { 1.24 0.10 |
| Sulphite Method | Unbleached pulp | { mg % | { 286.00 16.10 | { 56.55 72.40 | { 69.69 81.00 | { 5.42 18.00 | { 4.91 15.50 | { 1.10 61.40 | { — — | { 3.63 16.10 | { 8.37 0.80 |
| | Bleached pulp | { mg % | { 241.00 13.60 | { 142.81 66.10 | { 45.25 52.60 | { 3.95 13.10 | { 4.02 12.70 | { 16.21 94.30 | { — — | { 3.54 42.00 | { 7.54 0.70 |
| | α -Cellulose | { mg % | { 195.00 11.80 | { 124.11 57.40 | { 36.34 42.20 | { 1.71 5.60 | { 1.96 5.20 | { 15.93 92.80 | { — — | { 0.29 1.70 | { 0.87 0.80 |

TABLE III.

| Components Methods and kind | | Ash (%) | Pentosan (%) | Cu- Index | Relative Viscosity | “ α - Cellu- lose” | Unbleached pulp | |
|------------------------------------|---------------------|------------|-----------------|--------------|-----------------------|---------------------------------|--|---------------|
| | | | | | | | Alcohol Benzol Ext. Matter (%) | Lignin (%) |
| Nitric acid Method | Blea. pulp | 0.52 | 9.86 | 1.45 | 2.04 | 80.00 | 0.12 | 2.97 |
| | α -Cellulose | 0.21 | 6.28 | 1.39 | 2.14 | *(73.72) | | |
| Caustic soda Method | Blea. pulp | 1.11 | 7.20 | 1.41 | 1.72 | 86.64 | 0.34 | 10.23 |
| | α -Cellulose | 0.43 | 5.78 | 0.70 | 2.84 | (80.88) | | |
| Sulphate Method | Blea. pulp | 0.43 | 12.01 | 1.04 | 2.39 | 87.61 | 0.29 | 4.00 |
| | α -Cellulose | 0.28 | 9.43 | 0.75 | 2.53 | (78.18) | | |
| Chlorination Method | Blea. pulp | 0.67 | 10.33 | 2.14 | 2.28 | 87.08 | 0.68 | 2.36 |
| | α -Cellulose | 0.41 | 10.62 | 1.49 | 3.34 | (76.46) | | |
| Sulphite Method | Blea. pulp | 0.71 | 12.47 | 2.28 | 1.52 | 92.02 | 0.57 | 3.45 |
| | α -Cellulose | 0.33 | 9.38 | 1.54 | 2.64 | (82.64) | | |
| Mean | Blea. pulp | 0.70 | 10.37 | 1.66 | 1.99 | 86.68 | 0.40 | 4.60 |
| | α -Cellulose | 0.33 | 8.29 | 1.17 | 2.69 | (79.17) | | |

* N.B. α -Cellulose without Pentosan and ash.

Experimentelle Untersuchungen über die Wirkung von Radium und Röntgen-Strahlen auf die Gärungs-mikroorganismen. (III. Mitteil.)

Über die Bedingung der Citronensäuregärung durch
Asp. niger Radiumrasse III.

(SS. 761~771)

Von M. SIMO.

(The Institute of Research on Chemical Industry, Government-General of
Taiwan, Japan; Eingegangen am 2. 6. 1940.)

Blätteralkohol. IV. Mitteil.⁽¹⁾

Das *trans*- und *cis*-Problem bei Blätteralkohol, dem natürlichen Hexen-3-ol-1.

(SS. 772~780)

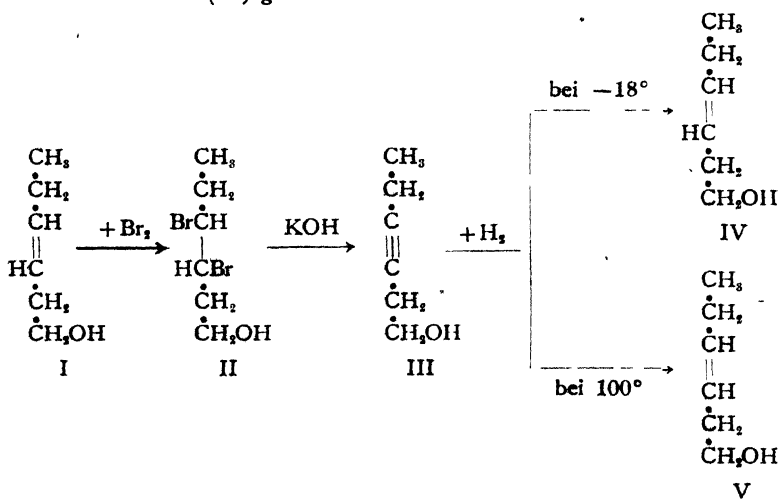
Von Sankiti TAKEI, Minoru ŌNO u. Kazuyosi SINOSAKI.

(Aus d. Agrikulturchem. Institut d. Universität Kyoto;
Eingegangen am 4. 7. 1940.)

In unseren früheren Mitteilungen⁽¹⁾⁽²⁾⁽³⁾ haben wir den in Pflanzenreich in freiem sowie gebundenem Zustand viel verbreitet vorkommenden Blätteralkohol, das β , γ -Hexenol, als *trans*-Hexen-3-ol-1 behandelt. Vor kurzem haben M. Stoll und A. Rouvé⁽⁴⁾ vom Methyläthylketon ausgehend mittels eines recht mühesamen Vorgehens Hexin-3-ol-1 hergestellt; daraus haben sie durch katalytische Hydrierung mit Palladium-Kolloid bei 21~23° Hexen-3-ol-1 gewonnen. Sie haben einfach angenommen, daß das in dieser Weise synthetisch erhaltene Hexen-3-ol-1 *cis*-Form besitzt. Sie haben nun das 3,5-Dinitrobenzoat dieses *cis*-Hexenols dargestellt und beobachtet, daß der Ester nach wiederholter Umkristallisation bei 45~46°, das Gemisch von diesem Ester mit 3,5-Dinitrobenzoat des natürlichen β , γ -Hexenols (Schmp. 48.5°) bei 47~48° schmilzt. Hierauf haben sie die Identität des Blätteralkohols mit dem synthetischen sogenannten *cis*-Hexen-3-ol-1 festgestellt. Unserer Anschauung nach ist bei diesem Experiment und seiner Deutung den schweizer Forschern ein großer Irrtum unterlaufen, welchem sie bei der katalytischen Hydrierung des so vorsichtig hergestellten Hexinols leichtsinnig verfallen sind.

Hexin-3-ol-1 (III) aus Blätteralkohol (I)

Wir haben neulich auf folgendem Wege aus dem Blätteralkohol (I) eine gute Ausbeute Hexin-3-ol-1 (III) gewonnen.



Das so erhaltene Hexinol (III) läßt sich bei 66~67/13 mm abdestillieren und besitzt einen schwachen eigentümlichen Geruch. Das Allophanat des Hexinols schmilzt bei 187°, das 3,5-Dinitrobenzoat bei 72° sowie das Anthrachinon- β -carbonat bei 129°. Bei Oxydationsabbau mittels Kaliumpermanganat gab es in quantitativer Ausbeute Propionsäure, die als *p*-Jodphenacyl-ester (Schmp. 97°) nachgewiesen wurde. Da wir glaubten, daß das Hexinol von Stoll mit unserem identisch sein müsse, haben wir nach seiner Vorschrift Hexin-3-ol-1 hergestellt und hieraus die obengenannten drei kristallinen Derivate abgeleitet. Durch Mischprobe der entsprechenden Präparate aus den beiden Hexinolen konnten wir ihre Identität bestätigen.

| | Schmelzpunkt des Hexinol-Derivats | | |
|---------------------------------|-----------------------------------|------------------------|-----------|
| | aus Blätteralkohol | nach Stoll u. Rouvé | Gemisch |
| Allophanat | 187° | 187° | 187° |
| 3,5-Dinitrobenzoat | 72° | 71° | 71°~72° |
| Anthrachinon- β -carbonat | 129° | 126° | 126°~128° |

Hexen-3-ol-1 (IV u. V) aus Hexin-3-ol-1 (III)

Bei der katalytischen Hydrierung einer dreifachen Bindung zur Doppelbindung sind die Temperaturbedingungen sehr wichtig. Wir haben zunächst Hexinol in Ätherlösung bei -18° und dann in Xylollosung bei 100° mittels Palladium-Bariumsulfat 1 Mol. Wasserstoff hydriert und die Reduktionsprodukte in kristallinen Derivate überführt. Die Schmelzpunkte der Derivate vergleichend, haben wir konstatiert, daß die bei der hohen Hydrierungstemperatur gewonnenen Hexenolderivate (V) bei einem niedrigeren Grade schmelzen als die entsprechenden der tieferen Temperatur erhaltenen (IV). Ferner wurde zugleich klar, daß die ersteren Schmelzpunkte mit denen der Derivate des synthetische aus Sorbinsäure-ester gewonnenen Hexenols übereinstimmen, die letzteren dagegen mit denen des Blätteralkohols.

| Hexen-3-ol-1 aus | Schmelzpunkte der Derivate | | |
|--------------------------------|----------------------------|-------------------------|-----------------------------------|
| | Allophanat | 3,5-Dinitro- benzoat | Anthrachinon β -carbonat |
| Hexinol bei -18° hydriert (IV) | 146° | 49° | 68° |
| Blätteralkohol (I) | 146° | 49° | 68°(2) |
| Hexinol bei 100° hydriert (V) | 143° | 28° | 50° |
| Sorbinsäure-ester mit Natrium | 143° | 28° | 50°(?) |

Man kann hier nach bei 1 Mol. Wasserstoffanlagerung an dreifacher Bindung nur durch Änderung der Reaktionstemperatur nach Belieben zwei Raum-Isomeren gewinnen. In unserem Institut haben Herr Prof. Y. Inoue und Herr H. Yukawa aus Stearolsäure durch 1 Mol. Hydrierung mit Platinschwarz bei -20° Elaidinsäure (*trans*-Form) und bei 100° Olsäure (*cis*-Form) in guter Ausbeute hergestellt.

Wir dürfen auf Grund obiger Ergebnisse wohl zu recht annehmen, daß in der Natur vorkommender Blätteralkohol, Hexen-3-ol-1, zur *trans*-Form gehört und er auch durch katalytische Hydrierung des Hexin-3-ol-1 bei tiefer Temperatur synthetisch

gewonnen werden kann. Ebenso läßt sich das *cis*-Hexen-3-ol-1, das Raum-Isomer von Blätteralkohol, synthetisch aus Sorbinsäure-ester durch Reduktion mit Natrium sowie aus Hexin-3-ol-1 durch Hydrierung bei hoher Temperatur erhalten. Wenn daher die 1 Mol. Hydrierung des Hexinols bei mittlerer Temperatur durchgeführt wird, so muss man ein Gemisch von *cis*- und *trans*-Hexen-3-ol-1 gewinnen. Bei 50° haben wir das Hexinol mit Palladium-Bariumsulfat 1 Mol. Hydrierung untersucht und das Reaktionsprodukt in 3,5-Dinitrobenzoat überführt; dabei haben wir zuerst ein Kristallgemisch von dem Schmelzpunkte 38~42°, der nach wiederholtem Umlosen aus Äthanol bis auf 48° erhöht wurde, erhalten. Aus dem Filtrat des Kristallgemisches nach dem Eindampfen und der Eiskühlung schied sich wieder eine Kristallmasse ab, die nach Umkristallisierung aus Petroläther bei 28° schmilzt. Hierauf geht ganz klar hervor, daß Stoll und Mitarbeiter in ihre obengenannten Arbeit nur das höherschmelzende Kristallgemisch erfaßt haben und das Filtrat unberücksichtigt gelassen haben.

Wie wir früher geschrieben haben, läßt der Geruch bei beiden raumisomeren Hexenolen einen deutlichen Unterschied bemerken, was man bei den aus dem entsprechenden kristallinen Ester zurückgewonnenen Präparaten noch viel deutlicher erkennen kann. Unser reines synthetisches *trans*-Hexen-3-ol-1 (IV) riecht gerade wie der natürliche Blätteralkohol, also rein grünlich.⁽⁷⁾

(1) III, Mittell, J. Agr. Chem. Japan, 13, 193 (1939), C 1939, 3705

(2) I, Mittell, J. Agr. Chem. Japan, 14, 709 (1938), C 1938, 3696

(3) II, Mittell, J. Agr. Chem. Japan, 14, 717 (1938), C 1938, 3696

(4) S. Takei, T. Imaki u. Y. Tada B, 68, 953 (1935)

(5) Helv 21, 1542 (1938)

(6) In dieser Hinsicht haben sich die Schweizer wie folgt ausgedrückt

"L'odeur de notre hexénol, bien que très proche de celle du produit naturel, a tout de même une note nettement différente. Nous attribuons ce fait à certaines impuretés qui influencent l'odeur de produit naturel aussi bien que celle du produit synthétique" (Helv 21, 1544 (1938))

Studies on the Essential Oil of Formosan Black Tea.

(pp. 781~802)

By Ryo YAMAMOTO, Ken ITO, and Hassai TIN.

(Iaioku Imperial University, Received July 6, 1940)

(Part IV.)

Continuing the previous experiments, we studied the neutral substances which existed in the distillate, the boiling point of the distillate being at 55~92°C (4 mm.) and not containing sulphurous compounds.

The substances were 71.8 g. in weight and were 41% of all the neutral substances, and also they were the most important substances for the flavour of black tea.

After separating the alcohols by the usual method (phthalic acid anhydride)

we examined the ingredients of the alcohols and found that almost all of them were aromatic primary alcohols, chiefly benzylalcohol and phenylethylalcohol. They were identified as 3,5-dinitrobenzoic acid ester, α -naphthylurethan and phenylurethan.

As was reported previously linalool exists in this distillate. Besides these alcohols, phenylpropylalcohol and unknown secondary turpen alcohol seem to exist.

In the same way, geraniol was separated from the distillate, of which the boiling point was at $95\sim 112^{\circ}\text{C}$ (4 mm) and this was identified as 3,5-dinitrobenzoic acid ester but citronellol was not identified in any way.

The neutral substances, excepting the alcohols, were separated by trimethylborate instead of metallic sodium. And 2-acetyl-pyrrol was separated from the distillate as nitrogenous compounds (Cf. the sixth report).

The distillate which does not contain nitrogenous and sulphurous compounds with the boiling point at $65\sim 75^{\circ}\text{C}$ (6 mm) seemed to be chiefly furan compound and had the characteristic flavour of black tea but it could not be identified as a pure substance.

(Part V.)

The sulphurous compounds which exist in the essential oil of black tea are classified roughly into the following three.

- A. Gaseous compounds.
- B. Compounds with middle boiling point.
- C. Compounds with higher boiling point.

A. Gaseous compounds :—Gaseous compounds were produced in rich amount in the case of extracting essential oil by steam distillation. Very little quantity of methylmercaptan was detected which mixed with aldehydes of lower members and other gaseous compounds.

B. Sulphurous compounds with middle boiling point :—These compounds existed partially in each distillate in case the boiling point was not higher than 82°C (40 mm). But chiefly these were caught in the receiver cooled at the temperature of -60°C in the case of fractional distillation. In these distillates acid substance existed. This was produced as the result of natural decomposition after the distillation and seemed to be sulphonic acid. From the neutral part, the sulphonic acid being derived from it, sulphuric acid and methylsulphonic acid were obtained as oxidation products. The former seemed to be obtained from monomethylsulphide and the latter from dimethyl sulphide or methylmercaptan.

C. Sulphurous compounds with higher boiling point :—These compounds existed in the distillates in case the boiling point was $95\sim 144^{\circ}\text{C}$ (4 mm). Sulphonic acid and sulphuric acid were obtained as the result of natural decomposi-

tion. As the oxidation products from the neutral part, sulphuric acid and methylsulphonic acid were obtained. The original form of the decomposed substance was perhaps methylsulphonic acid ester. In both cases of (B) and (C) thiophenes did not exist. The sulphurous compounds described above were produced either from China tea or Assam tea and they are probably the common ingredient of tea leaves.

(Part VI.)

From the neutral part of the essential oil obtained from 1300 kg of Formosan black tea by steam distillation, 0.6 g of fine colourless needle crystals of nitrogenous compound was isolated in the distillate of 70~78°C (3 mm).

It melted at 92°C exactly and was easily soluble in hot water and in almost all organic solvents. It especially showed a red pine wood reaction and sublimableness. Considering the usual analytical data it agrees most nearly to C_6H_7ON as the molecular formula

Finally it was identified as 2-acetyl-pyrrol (methyl- α -pyrrolketon) comparing with the synthetic substance and furthermore pentabrom derivatives, derived from both were ascertained as the same substance.

Bulletin of the Agricultural Chemical Society of Japan.

ABSTRACTS

from

TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noted.)

Study of the Insecticidal Principle in the Smoke Produced by Combusting Insect Powder. (Part I.)

(pp. 803~805)

By Makoto NAGASE.

(Agricultural Chemical Department, Taihoku Imperial University, Taiwan;

Received July 10, 1940)

I caught the smoke produced by combusting insect powder, which is used for mosquito coil, with 80 % methanol and 20 % sulphuric acid solutions.

After dilution the solution was distilled by steam distillation and acids, phenols, and neutral substances were separated from the distillate and bases from the mother liquor by the usual method. The yields from 1 kg powder were as follows.

| | |
|---------------------|--------|
| Neutral substances | 17 g. |
| Phenolic substances | 7 g. |
| Acidic substances | 2.7 g. |
| Basic substances | 3.5 g. |

To examine the insecticidal power *Drosophila melanogaster* were put in a 10 L. bottle which contained 0.03 g. of each fraction and observed for 5 min., keeping the temperature at 20°C. The percentage of those which fell down to the bottom were as follows.

| | |
|---------------------|-------|
| Neutral substances | 100 % |
| Phenolic substances | 53 % |
| Acidic substances | 22 % |
| Basic substances | 0 % |

From the quantity and insecticidal power, it is clear that the neutral fraction is the most important principle. But the remaining parts are also considered to have supplementary actions.

(Part II.)

From the smoke produced by combusting 20 kg insect powder, 60 g of

phenolic substance was obtained.

By fractional distillation, it was separated into two fractions of 121°/65 mm and 140~148°/65 mm.

By making derivatives of benzoate and 3,5-dinitrobenzoate, the former was decided as phenol.

Also by making derivatives of 3,5-dinitrobenzoate and aryloxy acetic acid, the latter was decided as *o*-cresol and its homolog.

On the Soil Properties in the Transitional Region of Steppe- and Brown Forest Soil in Manchuria.

(pp. 809~812)

By R. KAWASHIMA and G. SUYAMA.

(Agricultural Chemical Laboratory, Kyushu Imperial University

Received July 8, 1940)

In the transitional region of steppe- and brown forest soils in the western suburbs of Harbin, various soil properties change systematically regarding the increase of humidity, as already known in other countries.

For example, in accordance with the increase of humidity, the pH-values and calcium carbonate contents diminish successively and the clay contents increase.

The silica-alumina ratio and exchange capacity of colloidal clay increase in order.

On the Thermobacterium Orla-Jensen.

(pp. 813~818)

By Hideo KATAGIRI and Kakuo KIIAHARA.

(Department of Agriculture, Kyoto Imperial University, Received July 8, 1940)

We have never succeeded in the isolation of granulated, thermophilic and *l*-lactic acid producing homo-fermenters which would be included in the thermobacterium-group named by Orla-Jensen.

In the present paper, four strains of thermobacteria were isolated with fresh milk and sour mash by selective cultivations at about 50°.

All these strains never attacked pentoses, mannitol or glycerol, while vigorous fermentations were always observed with maltose and sucrose.

However, with galactose, lactose, inulin and starch, very different fermentative power was observed among these strains. Thus, it will be seen in Table I that these thermobacteria were classified into two species; *Lactobacillus lactis* and *Lactobacillus delbrueckii*, according to their potency of milk coagulation and the kinds of fermentable sugars; lactose and galactose.

Table I. Classification of *Thermobacterium* Orlà-Jensen.

| No. of Bacteria | Isolated from | Milk coagulation | Lactose | Galactose | Inulin | Starch | Species |
|-----------------|---------------|------------------|---------|-----------|--------|--------|--|
| 520 | Milk | + | +++ | +++ | 0 | ± | <i>L. lactis</i> var <i>galactosus</i> |
| 521 | Milk | + | ++ | +++ | +++ | ± | <i>L. lactis</i> var <i>inulinus</i> |
| 615 | Mash | 0 | 0 | ± | 0 | + | <i>L. delbrückii</i> |
| 616 | Mash | 0 | 0 | ± | 0 | + | <i>L. delbrückii</i> |

The two strains of *L. lactis* would again divide into two varieties owing to the fermentability of galactose or inulin. It is interesting to note that starch was easily fermented by one of the *L. delbrückii*.

On a new Classification of Lactic Acid Bacteria.

(pp. 819~831)

By KAKUO KITAHARA.

(Department of Agriculture, Kyoto Imperial University, Received July 8, 1940.)

We succeeded in our laboratory under the guidance of Prof. Katagiri in isolating almost all the known species of lactic acid bacteria which had ever been recorded, moreover five new species of *Lactobacillus* and a motile lactic acid bacteria named *Bacterium caseolyticum* were isolated by us, as already mentioned in the previous papers.

In the present paper, very satisfactory classification is proposed when ten characteristic natures; (1) Gram's staining, (2) form, (3) catalase, (4) manner of fermentation of glucose, (5) racemase, (6) fermentable sugars, (7) reduction of nitrates, (8) production of mannitol or volatile acid, (9) liquefaction of gelatin, and motility, (10) habitat, were chosen among various kinds of factors, as is shown in Table I.

Table I. The key to the species of lactic acid bacteria.

- a Gram-positive
 - b Cocci
 - c Without catalase
 - d Decompose glucose in the 1st type of fermentation, $C_6H_{12}O_6 = 2C_3H_7O_3$
 - e Without racemase **streptococcus** (*d*-lactic acid formers)
 - f No action on maltose
 - g No action on sucrose *Sc cremoris* Orlà-Jensen
 - gg Acid in sucrose *Sc thermophilus* Orlà-Jensen
 - ff Acid in maltose
 - g. No action on sucrose *Sc lactis* (Lister) Löhnis
 - gg. Acid in sucrose
 - h No action on pentose *Sc lactis* var.
 - hh Acid in pentose and mannitol **Enterococcus**
 - i No action on glycerol, *Sc faecalis* Andrews & Horder

- ii 'Acid in glycerol,
 j. Gelatin not liquefied, *Se glycerinaeus* Orla-Jensen
 jj Gelatin liquefied *Se liquefaciens* Orla-Jensen
- ee With racemase **Pediococcus** (*dl*-~*dl*+*d*-lactic acid formers)
 f Acid in maltose *Po hennebergi* Sollied
 ff No action on maltose *Pr lindneri* Henneberg
- dd Decompose glucose in the 2nd type of fermentation;
 $C_6H_{12}O_6 = C_3H_6O_3 + C_2H_5OH + CO_2$ **Leuconostoc** (*l*-lactic acid formers)
 e No action on pentose *Leuc dextranicum* (Bejerinck) Hucker & Pederson
 ee Acid in pentose *Leuc mesenteroides* (Cienkowski) Van Tieghem
 f Produce mainly mannitol from fructose *Leuc mesenteroides* α
 ff, Produce mainly ethanol from fructose *Leuc mesenteroides* β
- cc With catalase **Tetracoccus** (*d*-lactic acid formers) *Te liquefaciens* Orla-Jensen
- bb Rods
 c Without catalase
 d Decompose glucose in the 1st type of fermentation **True Lactobacillus.**
 e Produce *l*-~*dl*-lactic acid
 f Acid in lactose (Habitat mainly animal materials) *L. lactis* Orla Jensen
 ff No action on lactose
 (Habitat mainly cereal materials) *L. delbrückii* (Leichmann) Holland
 ee Produce *dl*-~*d*-lactic acid
 f No action on pentose (Habitat mainly animal materials)
 g No action on maltose *I. bulgaricus* sp
 gg Acid in maltose
 h No action on sucrose *I. casei* (Orla-Jensen) Holland
 hh Acid in sucrose *L. acidophilus* (Moro) Holland
 ff Acid in pentose (Habitat mainly cereal materials)
 g Acid in arabinose
 h Acid in mannitol *L. plantarum* (Orla Jensen) Bergey et al
 hh No action on mannitol *L. sake* nov. sp
 gg No action on arabinose, acid in xylose *L. xylosus* nov. sp.
- dd Decompose glucose in the 2nd type of fermentation
Beta-Lactobacillus (*dl* lactic acid formers)
 c Acid in raffinose, produce mannitol from fructose or sucrose
 f Acid in arabinose *I. brevis* (Orla Jensen) Bergey et al α
 ff No action on arabinose
 g Acid in xylose *L. fermentum* Bejerinck, α
 gg No action on xylose *L. betedelbrückii* nov. sp
 ee No action on raffinose, produce ethanol from fructose
 f Acid in arabinose *L. brevis* β
 ff No action on arabinose, produce acid generally in xylose *L. fermentum* β
- cc With catalase **Wild-Lactobacillus** (*d* lactic acid formers)
 d Nitrates not reduced *L. thermophilus* Ayers & Johnson
 dd Nitrates reduced
 e. Volatile acid not produced *L. reuteri* nov. sp.
 ee Produce a large amount of volatile acid
 f Gelatin not liquefied, *L. caseus* nov. sp
 ff Gelatin liquefied *Bact. caseolyticum* nov. sp.
- aa Gram-negative **Escherichia** (*l*-lactic acid formers revealing the 3rd type of fermentation)
 $2C_6H_{12}O_6 + H_2O = 2C_3H_6O_3 + C_2H_5OH + CH_3COOH + 2CO_2 + 2H_2$
Esch. coli (Migula) Castellani & Chalmers

On the Retting of Vegetable Fibre Materials. (Part XIII.)

A Useful Aerobe for the Bacterial Retting of Jute Fibre Materials.

(pp. 832~834)

By Hideo KATAGIRI and Tosio NAKAHAMA.

(Department of Agriculture, Kyoto Imperial University; Received July 13, 1940.)

An effective retting of jute fibre materials was attained by a Gram-positive, spore-bearing, non-motile bacillus among eleven aerobes isolated from the retting vats.

The useful bacillus revealed similar cultural characteristics to *Bacillus fulminans* Schrire and Greenfield. However, some physiological natures were found to be quite different, since indol was never produced, nitrate was not reduced and blood serum was not liquefied by the useful bacillus.

Therefore the bacillus was concluded to be a new species, and it was named *Bacillus cordhorus*.

Exchangeable Calcium and Magnesium of Soils in Tyōsen. III.

(pp. 835~844)

By Mitsu-Hideo.

(Agricultural Experiment Station, Government General of Tyōsen,

Received June 6, 1940.)

Nutritive-value of Extracted Perilla-Cake as Fodder. I~II.

(pp. 845~848)

By Michio GOTŌ.

(Agricultural Chemical Laboratory, Tokyo Imperial University;

Received July 17, 1940.)

A Study on Bacteria of Korean Liquor Barm.

(pp. 849~860)

By Y. L. PAK, M. D.

(Keijo (Seoul) Chosen; Received July 3, 1940)

The Korean yeast (Noo-Rook) is a kind of barm that contains fermenting bacteria particular to Korean liquor (Sake). Some reports have already been made on the study of the yeast in this particular barm, yet there is no literature available regarding the bacteria contained in this barm.

The author has made a study of these bacteria and isolated 14 kinds through the medium of both aerobic and anaerobic cultures, and has determined their species from the study of their biological nature, fermenting actions, and also by the products found in the culture media. They are as follows:—

- | | | | | |
|-----|-------------|----------------------------------|---|---|
| 1. | Micrococcus | subflavescens, No. 1, var. K. C. | | |
| 2. | Mycoplana | bullate, No. 1, | " | " |
| 3. | " | " No. 2, | " | " |
| 4. | Bacillus | lentas, | " | " |
| 5. | " | repens, | " | " |
| 6. | Micrococcus | conglomeratus, | " | " |
| 7. | " | Subflavescens, No. 2, | " | " |
| 8. | " | varians, | " | " |
| 9. | Bacillus | ambiguus, No. 1, | " | " |
| 10. | " | " No. 2, | " | " |
| 11. | Micrococcus | epimetheus, | " | " |
| 12. | Erwina | citrimaculans, No. 1, | " | " |
| 13. | " | aroidea, | " | " |
| 14. | " | citrimaculans, No. 2, | " | " |

Über den Azeotropismus von Aethylalkohol, Kohlenwasserstoff und Wasser. (I Mitteilung.)

(SS. 861~875)

Von M. SIMO und T. AIZAWA.

(The Institute of Research on Chemical Industry, Government-General of Taiwan, Japan Received July 26, 1940.)

Über den Schleim von *Brasenia Schreberi*, Gmel. (1)

Die Zuckerarten des Schleims. Die Gallussaure in dieser Pflanze.

(SS. 876~880)

Von Hikonojō NAKAHARA.

(Agrikulturchem Laboratorium, Kaiserlich Universität, Tokio,
Eingegangen am 5. 8 1940.)

Brasenia Schreberi ist eine in alten Teichen oder Sumpfen in der Natur vorkommende Wasserpflanze, deren Blütenknospen und junge Blätter mit Agar-Agar-artigem Schleime umhüllt sind. Im Sommer werden die jungen zarten Blätter gepflückt und hier in Japan als Zuspense verwendet.

Zur Analyse wird der Schleim durch Erhitzen im Autoklav verflüssigt. Nach Abkühlen wird die Flüssigkeit mit dem Gemisch einer Kupferlösung und einer Seignettesalzlösung versetzt, das bei der Zuckerbestimmung nach Bertrand erforderlich ist, und sofort scheidet sich ein flockiger Niederschlag einer Kupferver-

bindung aus, den man durch ein leinenes Tuch kolt. Den Ruckstand behandelt man wiederholt mit Salzsaure-Alkohol und nach dem Trocknen stellt er sich als ein weißes Pulver mit geringem Aschenghalt dar.

Die Analyse dieses Pulvers ergibt folgende Zahlen ;

| | |
|--------------------------|---------|
| Galakturonsäure Anhydrid | 22.00 % |
| Galaktan | 42.44 % |
| Mannan | 14.80 % |
| Rhamnosan | 11.82 % |
| Araban | 7.75 % |

Nachtraglich wird hier mitgeteilt, daß in dieser Pflanze freie Gallussaure vorhanden ist.

Die Jodometrische Furfurolbestimmung. (II. Mitteilung.)

(SS. 881~885)

Matsukitiro HAMADA und Kazuyuki MAEKAWA.

(Aus dem Agriculturchemischen Institut der Kaiserlichen Kyushu-Universität in Fukuoka; Eingegangen am 10. Juli 1940.)

On a Carbohydrase Acting on the Mucilage from *Chondrus ocellatus* Holmes. (II.)

Relations of the Enzyme to Inulase, Pectinase and Gelase.

(pp. 886~890)

By T. MORI.

(Department of Agriculture, Tokyo Imperial University; Received July 26, 1940.)

On the Fixation of Sericin of Raw Silk (Part IV.) Fixation by Basic Potassium Oxalatochromiate.

(pp. 891~894)

By Masami OKU and Zirô HIROSE.

(From the Fibre Chemical Laboratory, Ueda Imperial College of Sericulture and Silk Industry; Received July 26, 1940.)

In this report we have studied the influence of basic potassium oxalatochromiate solution upon the fixation of sericin of raw silk.

The experimental results were summarised as follows:—

1) The mode of adsorption of anionic chromium complex ion by α - and β -sericin when they were treated with basic potassium oxalatochromiate solution coincides completely with the formula of Freundlich's adsorption isotherm.

2) α -Sericin absorbs much more chromium of anionic form than does β -sericin.

3) Sericin could be precipitated almost quantitatively from its sol state by basic potassium oxalatochromate solution in the region of pH 4.7.

**(Part V.) Some Experiments accounting for the Theories of
Fixation by Formaldehyde.**

(pp. 895~897)

By Masami OKU

There have been proposed many theories about chemical reactions between proteins and formaldehyde. The theories of fixation of sericin by formaldehyde should also be attributed to those which were presumed from the point of view of the studies of protein. These theories can be summarised into two classes:— (1) the formation of methylene compound, (2) the formation of addition compounds by the reaction of amino group of protein by formaldehyde. In the former case, the reaction should be carried out through condensation, splitting some molecules of water.

In this experiment I have fixed sericin by formaldehyde mixed with hydrochloric acid or sulphuric acid of certain definite concentration which acts as dehydrating agent. The degree of fixation of sericin attained its maximum point when 0.5 % formaldehyde with 2.0 % HCl or H_2SO_4 was used. When fixed with formalin alone, the degree of fixation was considerably inferior.

From this experiment the fixation of the sericin of raw silk by formaldehyde should be attributed to the formation of methylene compound through the condensation reaction between amino-groups of sericin and formaldehyde. But the structure of methylene compound thus formed could not be determined in this experiment.

On the Stimulant for Cane Sugar Formation in Plants (VII.)

(pp. 898~900)

By Tetutarō TADOKORO and Masao NISIDA.

(Hokkaido Imperial University, Received August 7, 1940)

**Über den Proteingehalt des Getreide-chennichs unter
den verschiedenen Kulturumständen.**

(SS. 901~904)

Von Tetujirō OHARA.

(Tokyo Nogyō Kyōiku Senmongakkō, Eingegangen am 12 8 1940.)

Bulletin of the Agricultural Chemical Society of Japan.

ABSTRACTS

from

TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noted.)

Enzymatic Studies on Exuvial Fluid of *Bombyx Mori* L. (Silkworm)

(pp. 905~909)

Part I. Detection of the Enzymes in the Exuvial Fluid.

By Yasuji HAMAMURA, Senji IIDA and Minoru OTUKA.

(Kyoto Kōfō Sansi Gakkō; Received August 15, 1940)

We found protease, invertase and amylase in the exuvial fluid of silkworms and did not find lipase and tyrosinase. The exuvial fluid has hitherto been believed to exert only mechanical action at moulting, but our detection of enzymes therein suggests that the exuvial fluid acts not only mechanically but also enzymatically on an inner part of the old skin of silkworm at moulting.

Part II. On the Chitinase.

By Yasuji HAMAMURA and Yasusuke KANEHARA.

We found chitinase in the exuvial fluid and glucosamine in the water extract of the exuvia of silkworm. This fact shows that the chitinase in exuvial fluid acts on the chitin material of the exuvia at moulting.

The optimum pH of the chitinase action was found to be 8.2 and the optimum temperature 50°C.

Zur Kenntnis von 6-Nitro-derivaten des Sterins.

(Zuckerrohrwachs. VI. Mitteilung.)

(SS. 910~916)

Von T. MITUI.

(Aus d. Agrikulturchem. Laboratorium der Kaiserl. Universität Kyoto;

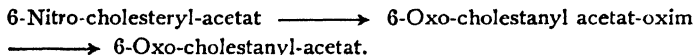
Eingegangen am 18. 8. 1910)

Reduktion von 6-Nitro-steroid.

Nach Windaus¹⁾ wird 6-Nitro-cholesteryl-acetat mittels Zinkstaub und Eisessig unmittelbar in 6-Oxo-cholestanyl-acetat reduziert.

Es ist dem Verf. gelungen, das Zwischenprodukt dieser Reaktion zu erhalten. Durch Behandlung mit Zinkstaub und Äther Eisessig (1:1) eragab 6-Nitro-cholesteryl-acetat eine Substanz vom Schmp. 199°, die bei weiterer Reduktion mit Zinkstaub und Eisessig in 6-Oxo-cholestanyl-acetat (Schmp. 128°; *p*-Nitro-phenylhydrazon: Schmp. 145°) übergeführt wurde.

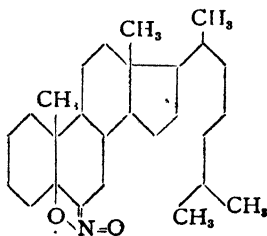
Aus 6-Oxo-cholestanyl-acetat wurde mit Hydroxylamin sein Oxim hergestellt, das bei 200° schmolz und mit dem oben gewonnenen Zwischenprodukt identisch war. Nun ist es klar geworden, daß die Reaktion wie folgt verlaufen ist.



Bei der Reduktion des 6 Nitro-sitosteryl-acetats sowie des 6-Nitro stigmasteryl-acetats wurde ganz analog als Zwischenprodukt das Oxim des 6-Oxo-sitostanyl-acetats (Schmp. 136°) bzw. des 6-Oxo-stigmastanyl-acetats (Schmp. 172°) erhalten.

Isomerisieren von 6-Nitro-steroid mittels Alkali.

Wenn man 6-Nitro-cholesten mit 5% Methanol KOH oder 5% Na-Methylat behandelt, so geht es in eine alkalilösliche Substanz über. Der beim Ansauern des Reaktionsgemisches auftretende Niederschlag wurde aus Methanol umkrystallisiert, dabei schied sich eine Substanz vom Schmp. 113° aus. Die Analyse dieser Substanz erbringt die Formel $C_{27}H_{46}O_2N$, ist also ein Isomer des Ausgangsstoffes. Ihren Eigenschaften nach muß sie die folgende Struktur besitzen.



Dieselbe Substanz kann man aus 3 Chlor-6-nitro-cholesten durch die gleiche Behandlung gewinnen.

Aus 6-Nitro-cholesteryl-acetat sowie aus 6-Nitro-cholesteryl-propionat durch Alkali-Behandlung wurde eine analoge Substanz vom Schmp. 152° erhalten, deren Derivate sind:

| | | | |
|---------------------------|--------|-------|----------------------|
| Acetat | Schmp. | 96.5° | $C_{29}H_{47}O_2N$ |
| Benzoat | Schmp. | 175° | $C_{41}H_{59}O_2N$ |
| <i>m</i> -Dinitro-benzoat | Schmp. | 158° | $C_{31}H_{47}O_6N_2$ |

Aus 6-Nitro stigmasteryl-acetat durch Alkali-Behandlung wurde die Substanz vom Schmp. 91~93° gewonnen.

Schrifttum.

Windaus: Ber., 36, 3754 (1903).

Oxydationsversuche mit Zuckerrohrsitosterin II.

(Zuckerrohrwachs. VII. Mitteilung.)

(SS. 917~492)

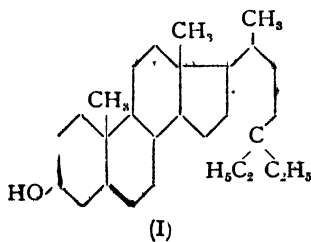
Von T. MITUI.

(Aus d. Agrikulturchem. Laboratorium der Kaiserl. Universität Kyoto;
Eingegangen am 18. 8. 1940.)

Wie in der II. Mitteilung berichtet wurde, hat der Verf. aus dem Oxydationsprodukt des Zuckerrohrsitosteryl-acetat-dibromids ein neues "Oxyketon" (Schmp. 114°) gewonnen. Nach weiteren Untersuchungen konnte er seine Struktur sicher stellen.

Durch die Clemmensen-Reduktion des Oxyketons hat der Verf. eine Oxyverbindung vom Schmp. 132° (Acetat: Schmp. 120°) gewonnen und als 3-Oxy-nor-sitosten angenommen. Inzwischen hat er 3-Oxy-nor-sitosten aus 3-Oxy-cholensaure auf folgende Weise synthetisiert.

3 Oxy- Δ^5 -cholensaure \rightarrow 3-Acetoxy-cholensaure-methylester — Diäthyl-Mg-J \rightarrow 3-Oxy-cholensaure-diäthyl-carbinol (Schmp. 160~163°; Dichlorid: Schmp. 116° \rightarrow Nor-sitosten: Schmp. 66~67°) — Essig-anhydrid bei 0° \rightarrow 3-Acetoxy-cholensaure-diäthyl-carbinol (Schmp. 129.5° — Essig-anhydrid bei 100° \rightarrow 3-Acetoxy- $\Delta^{5,6}$ -nor-sitostadien: Schmp. 117°) — Thionylchlorid \rightarrow 3-Acetoxy-cholensaure-diäthyl-carbinol-chlorid (Schmp. 130.5°) — Na-*n*-Propylat \rightarrow 3-Oxy-nor-sitosten (I) (Schmp. 134.5°; Acetat: Schmp. 137°)

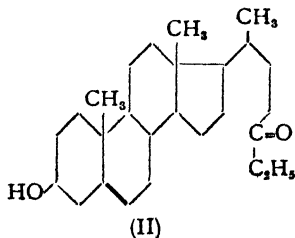


— Pt $O_2 + 1$ Mol $H_2 \rightarrow$ 3-Oxy-nor-sitostan
(Schmp. 131~132°; Acetat: Schmp. 131°)

Beim Vergleich dieses synth. 3-Oxy-nor-sitosten mit dem Reduktionsprodukt des Oxyketons ergab sich, daß die beiden Substanzen nicht identisch sind.

Hiermit wurde 3-Oxy-nor-cholesten-24-on und sein Derivat aus 3-Oxy-choleensäure hergestellt.

3-Acetoxy-choleensäure $\xrightarrow{\text{Thionylchlorid}}$ 3-Acetoxy-choleensäure-chlorid $\xrightarrow{\text{NH}_4\text{OH}}$ 3-Acetoxy-choleensäure-amid (Schmp. 210~212°) $\xrightarrow{\text{Diläthyl} \cdot \text{Mg} \cdot \text{J}}$ 3-Oxy-nor-cholesten-24-on (II) (Schmp. 114° bei 90° sint.; Acetat: Schmp. 167~168°) $\xrightarrow{\text{Clemmensen-Reduktion}}$ 3-Oxy-nor-cholesten (Schmp. 132°; Acetat: 120°)



Durch eine Mischschmelzprobe dieses synth. 3-Oxy-nor-cholesten-24-ons mit dem oben erwähnten neuen Oxyketon konnte ihre Identität bestätigt werden. Weiter wurde 3-Oxy-nor-cholesten durch die Clemmensen-Reduktion des synth. 3-Oxy-nor-cholesten-24-ons erhalten, das auch mit dem Reduktionsprodukt des aus Sitosteryl-acetat-dibromids erhaltenen neuen Oxyketons identifiziert wurde. Dieses 3-Oxy-nor-cholesten wurde auch noch aus 3-Oxy-nor-cholesten-25-on durch die Clemmensen-Reduktion erhalten.

Auf Grund dieser Ergebnisse wurde festgestellt, daß die Struktur des neuen Oxyketons vom Schmp. 114.6° zweifellos 3-Oxy-nor-cholesten-24-on (II) ist.

The Physiological and Chemical Function of Potassium in Plants, with Special Reference to the Behavior of Potassium in Growth and Maturity.

(pp. 925~938)

By Kisaburo SHIBUYA and Takashi TORII.

(Taihoku Imperial University; Received August 7, 1940.)

Exchangeable Calcium and Magnesium of Soils in Tyōsen. IV.

(pp. 933~948)

By MISU-HIDEO.

(Agricultural Experiment Station, Government General of Tyōsen; Received June 6, 1940.)

On the Selection of Grape Varieties for Wine Making. (Part 3.)

(pp. 949~962)

By Zenbei KAWAKAMI and Takasi HUKINBARA.

(Iwanohara Vineyard; Received August 26, 1940.)

The present study is the continuation of previous work reported by H. Kawakami and S. Masumiya and Z. Kawakami and T. Hasegawa.

The grapes used in the present experiments are known varieties and crosses of European and American origin besides new crosses obtained by Z. Kawakami in Iwanohara vineyard, Niigata Prefecture. They are as follows:—

a) Twenty-six known varieties of foreign and three Japanese native varieties are as follows:—

| | | |
|----------------|--------------------------------|----------------------|
| Hartford. | Campbell Early. | Unknown Spec. No. 3. |
| Big Extra. | Telegraph. | Cottage. |
| Zinfandel. | Cot à que Verte. | Carman. |
| Bailey. | Beacon. | Ives. |
| W. B. Munson. | Mills. | Concord. |
| Hybrid France. | Aomori and Sibu native grapes. | Jack. |
| Mataro. | Merlot. | Muscat Hamberg. |
| Herbement. | Unkown Spec. No. 1. | Niagara. |
| Perry. | Chasselas De Fontainebleau. | Gold Queen. |
| Sanjyaku. | Kôsu. | |

b) Thirty-five Z. Kawakami's new crosses are as follows:—

| | |
|--|---|
| No. 3 Big Extra. | No. 7 Extra Folle. |
| No. 55 Bailey Alicante A. | No. 56 Bailey Alicante B. |
| No. 69 Beacon Alicante. | No. 1 Bailey x Zinfandel. |
| No. 2682 Campbell Early x Highland. | No. 3986 Muscat Bailey A. |
| No. 4021 Bailey x Muscat Hamberg. | No. 4031 Muscat Bailey B. |
| No. 4083 Bailey x Muscat Hamberg. | No. 4131 Black Queen. |
| No. 4176 Bailey x Chasselas Cioutat. | No. 4183 Bailey x Muscat Hamberg. |
| No. 5778 Adirondack x No. 7 Extra Folle. | |
| No. 5788 Adirondack x No. 7 Extra Folle. | |
| No. 7709 Campbell Early x No. 7 Extra Folle. | |
| No. 7431 Carman Alicante. | No. 7788 Beacon x No. 56 Bailey Alicante B. |
| No. 7791 Beacon x No. 56 Bailey Alicante B. | |
| No. 7852 Black Hamberg x No. 56 Bailey Alicante B. | |
| No. 7875 Beacon x Folle Noire. | No. 7879 Bailey x Folle Noire. |
| No. 7882 Bailey x Folle Noire. | No. 7889 Bailey Alicante B x Beacon. |
| No. 385 Niagara x Brilliant. | No. 413 Rose Queen. |
| No. 4123 Bailey x Queen. | No. 4123 Gold Queen. |
| No. 4126 Bailey x Golden Queen. | No. 4600 Verderlho x Golden Chasselas. |
| No. 6421 Red Millennium. | No. 6952 Lady Washington x Sanjyaku. |
| Rose Cioutat. | White Bailey. |

Z. Kawakami's new crosses are generally better than the existing varieties in colour, but require more investigation as to taste and fragrance.

(1) H. Kawakami and S. Masumiya: This Jour., 14, 1437 (1938).

(2) Z. Kawakami and T. Hasegawa: This Jour., 13, 1149 (1939).

On Ascorbic Acid Formation in Plant and Animal Bodies. VI.

(pp. 963~964)

By Tetutaro TADOKORO and Masao NISIDA.

(Hokkaido Imperial University; Received September 5, 1941)

Experimentelle Untersuchungen über die Wirkung von Radium und Röntgenstrahlen auf die Gärungsmikroorganismen.

(SS. 935~978)

Von Mituo SIMO.

(The Institute of Research on Chemical Industry, Government-General of Taiwan,

Received September 16, 1940)

On the Pentose-fermenting Lactic Acid Bacteria.

(pp. 979~984)

By Mamoru IWASAKI.

(Agricultural Chemical Laboratory, Tokyo Imperial University;

Received Sept. 12, 1940.)

Two new varieties of lactic acid bacteria, which ferment xylose vigorously, forming lactic and acetic acids in the yields of 85~96% against the pentose used, were isolated. The ratio between the quantities of both acids produced was lactic : acetic = 58~59 : 41~42. Various conditions for the industrial application of the fermentation were studied. The bacteria were classified as follows:

Lactobacillus pentoaceticus var. *magnus*, nov. var.

Rods: Slender, 0.7~0.9 by 3~4 microns, occurring singly or in pairs. Non-motile. Spore not formed. Gram positive.

Broth: Carbohydrates necessary for growth. Bouillon or yeast extract: scanty. Wort or Koji-extract: Turbid within 2 days. Clears with somewhat slimy sediment and thin pellicle.

Gelatin: No liquefaction. Koji-extract or wort gelatin stab: Abundant development in stab and slight surface growth. Gas formation.

Agar: Koji-extract or wort agar slant: Narrow, whitish, somewhat translucent.

Litmus milk: Acid, without coagulation.

Nitrite reduction: negative.

Catalase not produced.

Temperature relations: Opt. for growth, 33~35°C. Opt. for acid formation, 31~33°C. Killed in 10 minutes at 65°C.

Acid formed from xylose, arabinose and fructose vigorously; from glucose, galactose and maltose moderately; from raffinose, mannose and lactose feebly. Saccharose, inulin and mannitol not fermented. Abundant volatile acid formed from xylose, arabinose and fructose.

Gas from fructose, glucose, mannose, galactose, raffinose and maltose. No or slight (if any) gas formation from arabinose and xylose.

Inactive lactic acid and acetic acid formed from xylose in the proportions of 58~59:41~42, and with the yields of 85~93% against the sugar consumed.

Mannitol formed from fructose.

Microaerophilic.

Source: Isolated from *Colocasia antiquorum*, Schott.

Lactobacillus mannitopoeus var. *fermentus*, nov. var.

Rods: Short, 0.6~0.7 by 1.2~1.5 microns, occurring sometimes singly, but mostly in pairs or chains. Non-motile. Spore not formed. Gram positive.

Broth: Carbohydrates necessary for growth. Bouillon or yeast extract: scanty. Koji extract or wort: Turbid with thin pericle and heavy sediment.

Gelatin: Not liquefied. Koji extract or wort gelatin stab: Good growth on surface as well as in stab. Gas formed.

Agar: Koji extract or wort agar: Filiform, milky white, somewhat shining.

Litmus milk: acid, without coagulation.

Nitrite reduction: negative.

Catalase not produced.

Temperature relations: Optimums for growth, 31~33°C; for acid formation, 28°C. Killed in 10 minutes at 60°C.

Acid formed from arabinose and xylose abundantly, from glucose, fructose, mannose, raffinose, saccharose, maltose, galactose, lactose and α -methyl-glucoside moderately. Abundant volatile acid from arabinose, xylose and lactose. Inulin and mannitol not fermented.

Abundant gas from fructose, glucose, raffinose, saccharose and maltose. No or slight (if any) gas from pentoses.

Inactive lactic acid and acetic acid formed from xylose in the proportion of 59:41, and with the yields of 90~96% against the sugar consumed.

Microaerophilic.

Source: Isolated from the fermented mash of Shao-hsing-chiu.

Studies on the Production of Acetone and Butanol by Fermentation.

(pp. 985~1006)

By Sinji DOI and Takeo YAMADA.

(Agricultural Chemical Laboratory, Tokyo Imperial University,

Received September 13, 1940.)

On the Chemical Composition of Loquat.

(pp. 1007~1011)

By Tasuku HIBINO.

(Chemical Laboratory, Hiroshima Higher School, Received September 16, 1940)

On the Production of 2,3-Butylene Glycol by Fermentation. A Supplement to Part I.

(A Method for the Industrial Utilization of Pentose).

(pp. 1012~1014)

By Kin-ichirô SAKAGUCHI, Mareyuki OHARA and Susumu KIKUTI.

(Agricultural Chemical Laboratory Tokyo Imperial University,

Received September 6, 1940)

In the previous paper the authors reported that 2,3-butylene glycol could be prepared technically through the fermentation process by the use of several strains of bacteria isolated by them. Saccharose, fructose and xylose have been used as raw materials in the present work. The yields of the glycol and ethyl alcohol against the sugars consumed have been found to be as follows:

| Sugars used | The yields of | | Sugars used | The yields of | |
|-------------|-----------------|---------------|-------------|-----------------|---------------|
| | Butylene glycol | Ethyl alcohol | | Butylene glycol | Ethyl alcohol |
| Xylose a | 27.03% | 15.65% | Fructose | 24.57% | 10.92% |
| b | 25.73 | 12.31 | Saccharose | 26.27 | 9.34 |
| c | 27.40 | 14.65 | Glucose | 29.51 | 12.23 |

From the results shown above, it is obvious that those sugars can also serve as raw materials for the production of the glycol. Various quantities of ethyl alcohol to the extent of about one half of these of the glycol are obtained as by-product.

Bulletin of the Agricultural Chemical Society of Japan.

TRANSACTIONS

Studies on Vitamin B₂ Complex.

VII.—The Rat Growth Factor in Liver Filtrate, with Evidence for its Multiple Nature.*

By Ume TANGE and Hide SASAKI.

(Received October 23, 1940)

In a previous paper,⁽¹⁾ one of us (U.T.) reported that the rat growth-promoting factor (or factors) in liver filtrate showed great resemblance in properties to factor W of Frost and Elvehjem⁽²⁾.

Recently Jukes⁽³⁾ and Wooley *et al.*⁽⁴⁾ have demonstrated that the "chick antidermatitis factor" is probably identical with pantothenic acid, which has been investigated by Williams and co-workers⁽⁵⁾. But Hoffer and Reichstein⁽⁶⁾ and Subbarow and Hitchings⁽⁷⁾ have also indicated that the rat filtrate factor is probably pantothenic acid. Edgar *et al.*⁽⁸⁾ have reported that their liver filtrate factor has at least three components.

Thus it has been shown that the so-called "filtrate factor" contains several components which are required for normal growth of rats.

This paper is concerned with the study of the rat growth-promoting substance contained in the different highly purified fractions obtained from liver filtrate.

EXPERIMENTAL.

Since we have found that sucrose is a better constituent for basal rations in the study of vitamin B₂ complex than are starches which contain some of the factors, the basal ration employed in the present experiments is changed in composition from that previously used, as follows:

| | |
|----------------------------------|-----|
| Purified fish protein | 20% |
| Sucrose (pharmacopeia Japonica), | 70% |
| Butter fat | 5 |
| Agar-agar | 1 |
| McCullum's salt mixture | 4 |

* This paper was presented at the Scientific Meeting of I. P. C. R., June 14, 1940.

Young rats weighing between 40 and 50 g. were placed on the basal diet supplemented daily with 20 γ of vitamin B₁ (synthetic B₁ chlor-hydrochloride), 30 γ of flavin,* 1 mg of nicotinic acid, and 2 drops weekly of biosterin as vitamins A and D. At the end of 3 weeks they were given daily, in addition to the above supplements, 20 γ of crystalline vitamin B₆ and an adequate amount of various liver fractions under investigation. The growth rate of the animals was observed for periods of at least six weeks.

METHODS.

Preparation of liver extract fractions.

3 liters (1 cc=10 g of fresh beef liver) of methanol extract⁽¹⁾ of raw liver, which has an acid reaction and is of a pale green-yellowish colour, was treated twice with 200 g portions of acid clay to remove flavin and inert substances which might interfere with further purification of the filtrate. The filtrate from acid clay adsorbates was neutralized with NaOH, and concentrated to viscous consistency in vacuo; then methanol was added until no further precipitate occurred. After standing several hours to allow the precipitate to settle, the clear solution was decanted, and the solvent was removed by distillation under reduced pressure. This methanol-soluble fraction was used as the starting material for all the experiments. A portion of this material was kept for assay. The feeding results are shown in Fig. 2 (a). The remaining portion was further evaporated under reduced pressure to syrupy consistency, and then repeatedly extracted with acetone containing 10% of water. The acetone extract was distilled under reduced pressure and water added to make 1 cc equal to 100 g of fresh liver (fraction A). Feeding the material at the level corresponding to 10 g of fresh liver brought about normal growth (Fig. 2 (b)).

From the results shown in Fig. 2(a) and (b), it appears evident that the active principle in methanol-soluble fraction is almost completely extracted with aqueous acetone.

The procedure employed for the concentration of the active substances is illustrated in Chart I.

The results shown in Fig. 3(a) and (b), indicate that charcoal eluate (fraction B) contains about one-half of the original activity, whereas charcoal filtrate, about one-fourth.

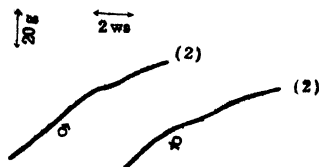


Fig. 1. Average growth curves of rats fed without liver filtrate, control ration. The figures in parentheses indicate the number of rats in the experiments.

* Flavins were prepared from dried egg white powder by the method described in a previous paper (Sc. Pap. I, P. C. R., 35 (1938), 59).

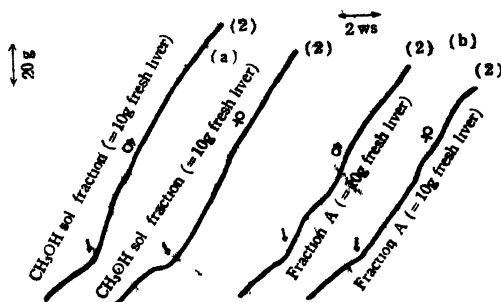
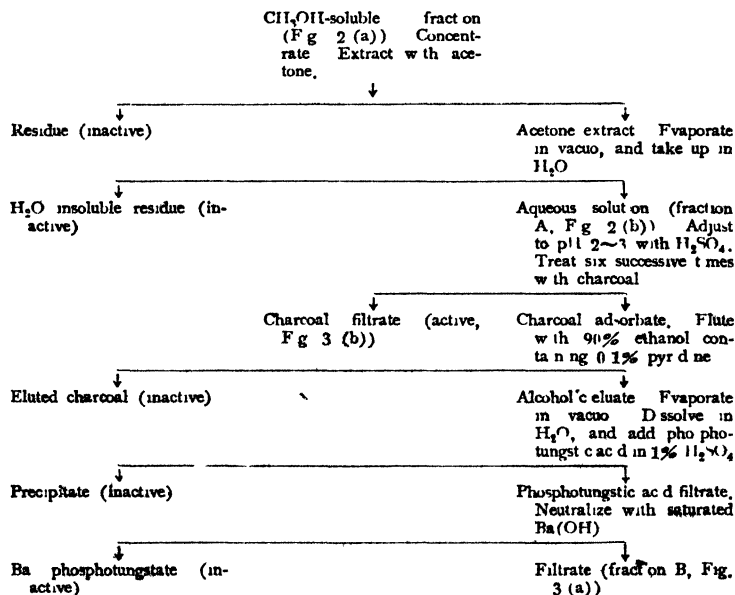


Fig 2 Average growth curves of rats on control ration, supplemented with CH_3OH soluble fraction (a) and with fraction A (b)

The figures in parentheses indicate the number of rats in the experiments

CHART I.

Steps in the concentration of the growth-promoting substances in liver extract.



During the course of the procedure some attempts were made to ascertain further properties of the "rat filtrate factor":

Mercury precipitation.— 50 cc of fraction A was diluted with water and a solution of about 20% mercury acetate added until no further precipitate occurred. After standing overnight, the precipitate was centrifuged, the filtrate was treated

with H_2S to remove mercury, and the sulphide filtered off. The filtrate was neutralized with $NaOH$, evaporated in vacuo, and dissolved in water and tested on rats.

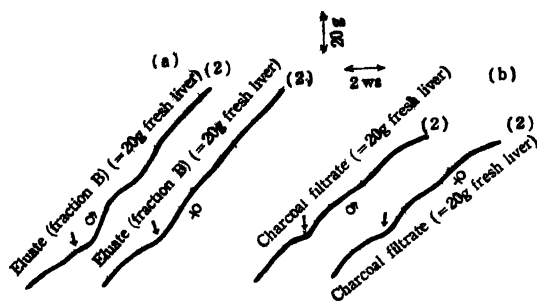


Fig. 3. Average growth curves of rats on control ration, supplemented with charcoal eluate (fraction B) (a), and with charcoal filtrate (b). The figures in parentheses indicate the number of rats in the experiments

The mercury precipitate was suspended in water acidified with H_2SO_4 , and Hg was removed by means of H_2S . The remaining material was treated as above and assayed on rats.

The mercury filtrate was found to have a growth-promoting activity approximately equal to that of fraction A, while the precipitate appeared inactive (Fig. 4).

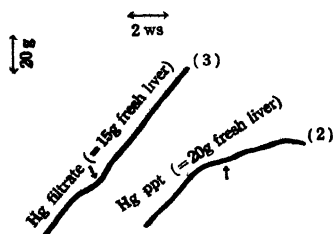


Fig. 4. Average growth curves of rats on control ration, supplemented with mercury filtrate or with the precipitate.

The figures in parentheses indicate the number of rats in the experiments.

Furthermore, it is indicated that pantothenic acid is very labile to alkali.

In our studies on this subject, the following procedure was undertaken.—Charcoal eluate (= 50 cc of fraction A) was dissolved in an equal volume of water, adjusted to pH 2.5 with H_2SO_4 and extracted continuously with ether for 72 hours. The ether was changed and the extraction continued for further 90 hours. Tested on rats, it was observed that the first ether extract contained about one-half of the activity present in the starting material, the second a very slight amount, and the residue about one-half.

A portion of the first fraction of the acid-ether extract was evaporated under

reduced pressure, and was dissolved in 5% NaOH. The mixture was heated on water-bath for 2 hours, cooled and neutralized with HCl. There was no appreciable activity in the material thus treated when fed at the level equal to 20 g (or more) fresh liver (Fig. 5). It was, therefore, assumed that pantothenic acid was destroyed in this treatment.

The acid-ether residue was also heated in the same manner as above in the presence of 5% NaOH. The neutralized substance showed slight growth activity when given to rats (Fig. 6).

Acetylation.— A portion (=50 cc of fraction A) of the filtrate, after precipitation with mercury acetate, was evaporated to dryness under reduced pressure and taken up in a mixture of 15 cc of pyridine and 65 cc of acetic anhydride. The solution was allowed to stand at room temperature for several days. The reaction mixture was evaporated in vacuo and the residue was nearly soluble in chloroform (the insoluble residue appeared to be inactive). Into the chloroform solution an equal volume of water was added and shaken, and the chloroform layer was separated from the aqueous layer.

Chloroform layer.— This was evaporated and hydrolyzed with *N*/10 sodium methoxide, standing at room temperature overnight, and then neutralized with HCl. This was concentrated under reduced pressure and prepared for assay.

Aqueous layer.— This was evaporated and hydrolyzed with *N*/10 sodium methoxide as above and tested on rats.

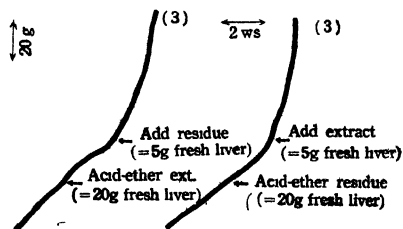


Fig 5 Growth curves showing the effects of acid-ether extract and residue supplements.

The figures in parentheses indicate the number of rats in the experiments.

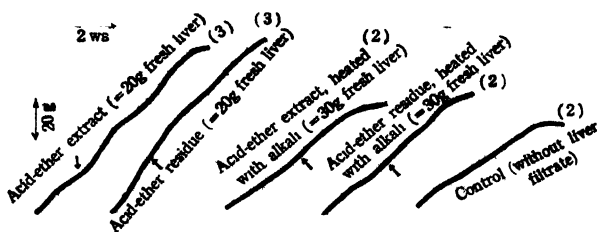


Fig 6. Growth curves showing the effect of acid-ether extract and residue, and of both fractions heated with alkali.

The figures in parentheses indicate the number of rats in the experiments.

The hydrolyzed fractions of both chloroform and aqueous layers stimulated the growth of rats as seen in Fig. 7, while the unhydrolyzed fraction produced only a very slight response.

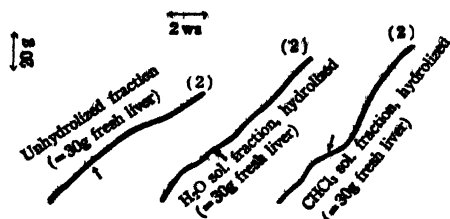


Fig. 7. Growth curves of rats on control ration, supplemented with acetate and its hydrolyzed fractions.

The figures in parentheses indicate the number of rats in the experiments.

DISCUSSION.

Recently several investigators⁽⁹⁾⁽¹⁰⁾⁽¹¹⁾ have reported evidences for the essential nature of pantothenic acid in the nutrition of the rat. In the concentration of pantothenic acid from the liver filtrate, acetylation and acid-ether extraction have been used as steps in the procedure. Wooley *et al.*⁽⁹⁾ have used this method in the concentration of the "chick antidermatitis factor" or pantothenic acid. Hoffer and Reichstein,⁽⁹⁾ and Subbarow and Hitchings⁽⁷⁾ have evidence indicating that pantothenic acid is the active component of their ether extract. Lunde and Kringstad⁽¹¹⁾ have stated that factor W is not extractable with acid-ether and that this factor can be differentiated from pantothenic acid.

We have separated two fractions by acid-ether extraction and believe that the factor in the ether extract is probably pantothenic acid and the factor in the residue is factor W of Frost and Elvehjem.⁽⁹⁾ The growth rate was significantly greater when both fractions were given together than when each fraction was administered alone. This supplementary effect of the acid-ether extract and the residue suggests that pantothenic acid is responsible for growth-promoting function of liver extract, but for the maximum growth at least one other factor (factor W?) is required. This finding agrees with the results of Hoffer and Reichstein,⁽⁹⁾ and of Black, Frost, and Elvehjem,⁽¹²⁾ working with liver filtrate. The typical growth response is shown in Fig. 5.

It should be remembered that our experiments have been confined to rats and we are therefore unable to demonstrate the identity of our factor with pantothenic acid, which is probably the "chick antidermatitis factor." However, our liver filtrate factor shows great similarity in properties to both pantothenic acid and the growth factor termed factor W. Final proof as to the relationship of these factors must await further study.

SUMMARY.

1. Procedures are described for the concentration of the rat filtrate factor complex.

2. This complex is not adsorbed by acid clay; it is, however, adsorbed by large amount of charcoal, from which the active substances are eluted with 90% ethanol containing 0.1% pyridine.

3. The factor (or factors) is not precipitated by either phosphotungstic acid or mercury acetate.

4. It is not inactivated by acetylation; mild hydrolysis of this material produces a good growth response, the unhydrolyzed substance, however, possesses only slight activity.

5. The supplementary effect of the acid-ether extract and residue suggests that in addition to pantothenic acid, the rat requires an additional factor (factor W?) for the normal growth.

We wish to express our deep gratitude to Prof. U. SUZUKI and Prof. B. SUZUKI for their advice and encouragement throughout this work. We gratefully acknowledge Dr. F. INUKAI's gift of a large amount of liver extract and are also indebted to Miss Sizuye OTSUKA for her willing help in feeding the animals and preparing the materials.

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ABSTRACTS

from

TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noted.)

On the Formation of *l*-Ethylene-Oxide- α , β -Dicarboxylic Acid by Moulds. Part V.

(pp. 1015~1016)

By Kin-ichiro SAKAGUCHI and Tatuitiro INOUE.

(Agricultural Chemical Laboratory, Tokyo Imperial University, Received October 23, 1940)

Acetic and *dl*-lactic acids were identified among the metabolic products from glucose by *Monilia formosa*⁽¹⁾ beside *l*-ethylene-oxide α -, β -dicarboxylic, citric and succinic acids which were already reported to be formed⁽²⁾. Fenton's reaction⁽³⁾ which is characteristic of tartaric acid, was also given by an ether insoluble residue, but the isolation of the acid responsible for the reaction was not accomplished.

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On Lactose-fermenting Yeast.

(pp. 1017~1037)

By Motokiti HONGŌ.

(Agricultural Chemical Laboratory, Tokyo Imperial University, Received September 19, 1940)

Über Nutzbarmachung des Vitamins C aus dem Pflanzenreich in Taiwan (IV. Mitteilung).

Über die Reaktionen zwischen Ascorbinsäure und MgO.

(SS. 1038~1040)

Von Ryo YAMATO und Takeshi HARA.

(Agrikulturchemisches Laboratorium, Taihoku Kaiserliche Universität, Taiwan,
Eingegangen am 9. 10. 1940)

Wir haben festgestellt, dass reine Ascorbinsäure in wässriger Lösung mit MgO im Molekulargewichtsverhältnis 1 : 0.5 zu dem Salz geführt werden kann, welches den Mg-Gehalt von 6.69 % zeigt (als $(C_6H_7O_6)_2Mg$ 6.49 %), $[\alpha]_D^{25} = +96.5^\circ$; und im Molekulargewichtsverhältnis 1 : 40 oder mehr zu einem unlos-

lichen Verbindungskörper geführt und von diesem Körper unter Zusatz von Saure wieder zu einer Lösung zurückgeführt werden kann. Von dieser Lösung isolierten wir die Krystalle der Ascorbinsäure.

Biochemical Studies on a Nutritional Yeast Preparation.

(pp. 1041~1044)

By Tetutaro TADOKORO and Naomoto TAKASUGI.

(Department of Science, Hokkaido Imperial University; Received October 3, 1940)

On the Cellulose Analysis and Bleaching Methods of Cellulose Materials. Part III.

Modified Method for New Cellulose Estimation
by Bleaching Powder.

(pp. 1045~1056)

By Sin-iti HONDA.

(Kyoto Imperial University, Received October 19, 1940)

In the previous papers the present author had modified Jenkins and Norman's cellulose estimation method with bleaching powder⁽¹⁾ and as to the original method, they reported that the bleaching powder procedure was more advantageous and excellent than the NaOCl procedure. These results were also shown in the previous paper.

The present author tried to omit the so-called neutral treatments with NaOCl and 3% Na₂SO₃. This idea is conformable with the experimental results of Norman and Shrikhande⁽²⁾, that hemicellulose as well as cellulose combined with lignin, and the neutral treatments with NaOCl and Na₂SO₃ was not effective for elimination of hemicellulose. In the present paper, it is shown that the elimination of neutral treatments gave no serious effects for the analytical purpose as shown in Table I. Thus the procedures of the analyses were much simplified.

Table I. Comparison of analysis by various methods with bakkoyanagi (*Salix Caprea* L). (Oven dry state.)

| Component | Method of chlorination Procedure of Analysis | Gaseous state, Modified procedure of Cross & Bevan's Method. | Liquid state (State of solution.) | | | |
|----------------------------|---|---|-----------------------------------|--------------------|---|--------------------|
| | | | NaOCl-Method (Jenkins & Norman's) | | Bleaching Powder Method (author's method) | |
| | | | Previous procedure | Improved procedure | Previous procedure | Improved procedure |
| Total cellulose (%) | | 54.95 | 47.68 ± 0.66 | 53.51 ± 0.41 | 55.69 ± 0.32 | 57.26 ± 0.66 |
| α-cellulose (ash-free) (%) | | 37.27 | 39.88 ± 0.16 | 39.39 ± 0.26 | 39.26 ± 0.41 | 39.55 ± 0.41 |

| | | | | | |
|-----------------------------|-------|------------------|------------------|------------------|------------------|
| In total cellulose;— | | | | | |
| α -cellulose (%) | 67.83 | 83.59 \pm 1.02 | 73.39 \pm 0.16 | 70.60 \pm 0.31 | 69.86 \pm 0.30 |
| α -cellulose ash (%) | — | 0.36 | 0.14 | 0.09 | 0.19 |
| β -cellulose (%) | 32.16 | 16.05 | 17.22 | 28.45 | 29.95 |
| γ -cellulose (%) | — | — | 9.03 | — | — |
| Number of chlorination, (1) | ? | 2N, 7A. | 5A | 2N, 3A. | 3A. |

(1) Notations are according to Jenkins and Norman.

The total cellulose content given by the modified method was increased by about 1.5 %, for example, 57.26 % instead of 55.69 % by Jenkins' method.

Such differences were considered to be due to the incomplete separation of hemicellulose from the total cellulose fraction, but for the practical purpose, especially for the paper pulp analysis, it may be sufficient.

The α -cellulose contents, which is important for rayon pulp analysis, considered with Jenkins' original method and thus the α -cellulose analysis, owing to the simplicity of the analytical process.

In the previous paper, comparisons of using 2 % Na_2SO_3 instead of 3 % in Jenkins' original method in every Na_2SO_3 treatment were given.

In the present paper, the writer applied 2 % Na_2SO_3 treatments, thus the difficulty of 3 % Na_2SO_3 solution treatment was avoided.

The results are shown in Table II.

Table II. Extraction of sodium sulphite solution by boiling with yulin sun (*Picea ajanensis* Fisch.). (Oven dry state.)

After 1 gram of sample refluxed with 2 % Na_2SO_3 10 minutes, filtered and dried, then analyzed the components

| | Original wood, (%) | Residue of treatment (based on the original wood) (%) | Extracted contents by 2 % Na_2SO_3 solution (%) |
|--|--------------------|---|---|
| | A | B | (A—B) |
| Moisture | 10.26 \pm 0.16 | — | — |
| Extracted contents by Na_2SO_3 | — | 6.28 \pm 0.01 | 6.28 |
| Lignin (1) | 28.61 \pm 0.13 | 27.76 \pm 0.05 | 1.05 |
| Methoxyl in lignin. | 16.78 \pm 0.40 | 15.25 \pm 0.05 | — |
| Pentosan | 12.09 \pm 0.09 | 11.48 \pm 0.10 | 0.61 |

(1) Lignin was estimated by the following procedure; Samples extracted with alcohol-benzene (1:1) mixture and hot water, hydrolyzed with 72 % H_2SO_4 in ice chest for 48 hours, then diluted to 3 % and refluxed 3 hours

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(Prof. Sukata's Laboratory, The Institute of Chemical Research, Kyoto Teikoku-Daigaku)

Researches on the Electrolytic Reduction Potentials of Organic Compounds. Part XXVIII.

The Quantitative Analyses of Sugars by the Polarographic Method.

(1). The fundamental experiments for the analyses of pentoses and pentosan.

(pp. 1057~1063)

By Isamu TACHI.

(Agricultural Chemical Institute, Kyoto Imperial University ;

Received October 2, 1940)

Pentoses and pentosan are able to be quantitatively determined by the polarographic method by means of the estimation of furfural derived from them. The author has investigated the relation between the concentration and the height of the reduction wave of furfural, because this is very important for the polarographic analysis. It was shown that if the height of wave was measured by the so-called tangent point method the relation was shown with a straight line which passed through the original point of the co-ordinates. It was revealed that furfural was quantitatively produced from xylose, which was taken as a sample of pentoses, when xylose was heated with sp. g. 1.060 HCl for 2~3 hours at 160°C.

Pentosan in a hard wood which was produced in Siam was measured by the polarographic method and the value agreed with the value obtained by the phloroglucide method.

On the Application of Hydrogen Peroxide for Brewing. (Part IX.)

On the Catalase of *Aspergillus oryzae*.

(pp. 1064~1070)

By Hisao MATUI.

(The Governmental Institute of Brewing, Takinogawa, Tokyo ;

Received September 20, 1940.)

The properties of the catalase excreted by *kozi* fungi (*Aspergillus oryzae*) were studied in culture medium and the results are summarized as follows:—

1. When *kozi* fungi are cultivated in *kozi* extract, the catalase action of the medium reaches the maximum in 7~10 days culture and then gradually decreases.
2. The inactivation of *kozi*-catalase first appeared when heated above 60°, suddenly becoming emphasized above 75°.
3. *Kozi*-catalase reacts with the monomolecular reaction at 1°, but at a higher temperature the velocity constant falls with the lapse of time.
4. When the temperature of the reaction mixture (0.008 *N* H₂O₂) is above 30°, the catalase is inactivated strikingly by H₂O₂ in it.
5. The optimum temperature for the catalase action is 30° or a little higher.
6. The optimum pH for the catalase action is about 7.

7. It has been observed that the natural salt (NaCl) added to culture medium (kozi extract) influences the formation of the catalase of *A. oryzae*; i. e., with the addition of NaCl in 2~5% the enzyme formation decreases, while in 7~10% it is increased.

8. If the kozi fungi are cultured on different substrata, these latter influence the catalase formation.

| Strains of <i>A. oryz.</i> | A | B | Strains of <i>A. oryz.</i> | A | B |
|----------------------------|------|------|----------------------------|-----|------|
| Kozi extract | 13 | 81.0 | Henneberg's medium | 9.5 | 61.0 |
| Pfiffer's medium | 57.3 | 4.6 | | | |

(Figures show the activity of the catalase in 30 days cultures)

9. The catalase is more effectively extracted from kozi (*A. oryzae* grown on steamed rice) with 0.25~0.5 % salt solution than with pure water, but if the salt content is over 0.5 %, the elution of the enzyme suddenly decreases.

10. When the catalase is extracted from kozi, the higher (up to 55°) the temperature, the more the enzyme is extracted.

11. The Taka-diestase includes the catalase of kozi fungus, and the optimum hydrogen ion concentration for this enzyme is about 7.

12. The diastase preparation prescribed by the Japan pharmacopoeia (malt diastase) contains little catalase.

On the Application of Hydrogen Peroxide for Brewing. Part X.

On the Catalase of Moulds and Yeasts.

(pp. 1071~1073)

By Hisao MATUI.

(The Governmental Institute of Brewing, Takinogawa, Tokyo;

Received September 29, 1940.)

The catalase of thirty-seven strains of moulds—*Aspergillus* (19), *Monascus* (1), *Penicillium* (5), *Rhizopus* (6), *Mucor* (4) & *Absidia* (2)—and five strains of yeast—*Saccharomyces saké*, *S. cerevisiae*, *S. ellipsoideus*, *Willia* & *Torula sanguinea*—was investigated.

1. Of all the moulds *A. oryzae* excretes a particularly large amount of catalase when it is cultured on kozi extract, and *A. flavus* and *A. melleus* rank next.

2. There is a relation between the catalase action of the culture medium and the age of culture; i. e., when the moulds like *A. oryzae*, which excrete comparatively large amount of enzyme, are cultured the catalase action of the culture medium reaches the maximum in 1~2 weeks and then declines gradually, while when the moulds which excrete a small quantity of enzyme are cultured,

the catalase action still increases little by little even after cultivation for 30 days.

3. Although yeasts also excrete catalase in the culture medium, the amount of the enzyme is far less than that of *A. oryzae*.

On the Fatty Oil of Awa (*Setarica itarica*, Beauv) Bran.

(pp. 1074~1076)

By Yoshikatsu MANO.

(The Institute of Scientific Research, Manchoukuo; Received October 22, 1940.)

Some of the values of the fatty oil were estimated.

Also the fatty acids of this oil were classified approximately as follows:—

| | | | |
|-------------------|--------------------------------------|--|-------------|
| Total fatty acids | { Solid fatty acids | | about 10.6% |
| | { Liquid fatty acids | | about 89.4% |
| | { Fatty acids of oleic acid series | | about 10.6% |
| | { Fatty acids of linolic acid series | | about 80.7% |

The unsaturated fatty acids were converted to their respective oxy-fatty acids and from the properties of these latter the identity of each original fatty acid was deduced.

On Xylitol. (I)

Preparation of Xylitol by Catalytic Reduction with Hydrogen
under Pressure and the Uses of Xylitol.

(pp. 1077~1079)

By Teijiro YABUTA and Kiyoshi Aso.

(Agricultural Chemical Laboratory, Tokyo Imperial University;
Received September 30, 1940.)

Exchangeable Calcium and Magnesium of Soils in Tyosen.

(pp. 1080~1088)

By Hideo MISU.

(Agricultural Experiments Station, Government General of Tyōsen; Received June 6, 1940.)

Untersuchungen über Vitamin in Obstsaffabrikaten. (II).

Einfluss des Unterschiedes der Klarung und der Lagerung
auf den Vitamin B₁-Gehalt in Apfelsinensaft.

(SS. 1089~1097)

Von Tyoten INAGAKI und Susumu OHASHI.

(Lebensmittelchemisches Forschungsinstitut der Meiji Zuckerindustrie;
Eingegangen am 19. 9. 1940.)

Es wurden Untersuchungen ausgeführt über den Vitamin B₁-Gehalt mit 4 Apfelsinensaftbuxen und 1 Apfelwein von inländischen Waren.

Nach der *p*-Aminoacetophenon-Methode mit dem Pulfrichphotometer kommt im frischen Apfelsinensaft ein Vitamin B₁-Gehalt von durchschnittlich 10.7 γ auf je 100 g.

Durch wiederholte Untersuchungen wurde die Abwesenheit von gebundenem Vitamin B₁ im frischen Apfelsinensaft bestätigt.

Weiter wurde die enzymatische Klarung von Apfelsinensaft unter besonderer Berücksichtigung der Filtrationsenzyme untersucht, sowie die Klarung von Apfelsinensaft reich an Vitamin B₁ und die beste Lagerungsmethode zur Aufbewahrung.

On the Vitamin Contents of Dried Mushrooms Produced in Manchoukuo.

(pp. 1098~1100)

By Hideo MIYAYOSHI and Kozo KAWAKAMI.

(The Institute of Scientific Research, Manchoukuo; Received October 22, 1940)

Certain vitamin and ergosterol contents of *Pleurotus serotinus* (Schrod) Fr., *Armillaria mella* (Vahl) Fr., etc, were estimated and the following results obtained:—

| | ^{γ} B ₁ γ per 100 g | B ₂ γ per 100 g | C γ per 100 g | Ergosterol gr. % |
|-----------------------------|--|--------------------------------------|-------------------------|---------------------|
| <i>Pleurotus serotinus</i> | 33.4 | 1292.7 | 25.312 | 0.250 |
| <i>Armillaria mella</i> | 8.0 | 52.5 | 11.237 | 0.300 |
| <i>Cortinellus Shittake</i> | — | 526.0 | 17.777 | 0.277 |

On the Denaturation of Sericin. (Part 2.)

Isoelectric Point of α -sericin.

(pp. 1101~1106)

By ZIRŌ HIROSE.

(Sericultural Research Laboratory of Gunze Raw Silk Mfg. Co. Ltd;

Received October 12, 1940.)

(1) INTRODUCTION.

In the previous paper, we studied denaturation of sericin retained in the raw cocoon layers, caused by boiling in hot water; and found denatured sericin (retained sericin) in cocoon layers took up more anionic chromate complex and minor cationic chromate complex than the original one, corresponding to the time of treatment. But in this treatment, 5~10 % of sericin was extracted from the raw cocoon layers. So we can easily imagine, that this difference of tanning capacities between retained (insoluble) and extracted (soluble) sericin fractions may be due to the modification of physico-chemical structures between both cases, and to the denaturation of sericin occurring in the process of cocoon boiling, and that isoelectric point of α -sericin in soluble and insoluble sericin fractions may have different tendencies according to the modification of their ionic structures.

In this paper we studied isoelectric point of α -sericin in soluble and insoluble sericin fractions. But in this and further reports, we mean α -sericin by one which can be obtained as precipitate when making pH of sericin sol 3.2~5.2, and β -sericin by one which can be obtained as precipitate from the filtrate of α -sericin by increasing the concentration of alcohol up to 50 %, adding ethanol to the filtrate.

(2) EXPERIMENTAL.

(A). The modification of isoelectric point of α -sericin in soluble and insoluble sericin fractions.

When sericin is extracted from the same raw cocoon layers by treating with boiling water for a short time repeatedly, water in each case being renewed, it is clear that soluble sericin fraction is extracted at the very beginning, and corresponding to the time of extraction, from soluble to insoluble fractions are being extracted. In this part we studied isoelectric point of α -sericin in soluble and insoluble fractions obtained according to the above idea. The procedure was as follows;—

45 gr. of raw cocoons, carefully freed from chrysalid, were extracted by boiling for 10 minutes in 3 l. of distilled water. The extraction was repeated 4 times, water in each case being renewed. The nitrogen contents in each extract was determined and compared with that of filtrate which was obtained by filtering off the precipitate caused by addition of the acetate mixture (final conc.—0.02 m) of various hydrogen ion concentration. The difference of the two values gives the amount of sericin precipitated, and the pH value where the highest precipitate was

formed was taken as the isoelectric point of α -sericin. Experimental result was as follows;

(Quantity of N is expressed in mg/200 cc.)

| Number of Extractions | Total Nitrogen | Kind of Sericin \ pH | 3.2 | 3.4 | 3.6 | 3.8 | 4.0 | 4.2 | 4.4 | 4.6 |
|-----------------------|----------------|----------------------|-------|-------|------------|------------|-------|-------------|-------------|-------|
| 1 | 29.12* | α -Sericin N. | 14.56 | 14.91 | 15.33 | 18.62 | 18.76 | 18.94 | 19.88 (max) | 17.15 |
| | | β -Sericin N. | 14.56 | 14.21 | 13.79 | 10.50 | 10.36 | 10.18 | 9.24 | 11.97 |
| 2 | 14.21* | α -Sericin N. | 9.59 | 9.66 | 9.67 | 9.98 | 10.17 | 10.22 (max) | 9.87 | 8.19 |
| | | β -Sericin N. | 4.90 | 4.83 | 4.82 | 4.51 | 4.32 | 4.27 | 4.62 | 6.30 |
| 3 | 6.09* | α -Sericin N. | 3.39 | 3.39 | 4.68 | 4.87 (max) | 4.27 | 3.39 | 3.37 | 3.35 |
| | | β -Sericin N. | 2.70 | 2.70 | 1.41 | 1.22 | 1.82 | 2.70 | 2.72 | 2.74 |
| 4 | 5.11* | α -Sericin N. | — | 3.57 | 3.71 (max) | 3.29 | 8.22 | 3.01 | — | — |
| | | β -Sericin N. | — | 1.54 | 1.40 | 1.82 | 1.89 | 2.10 | — | — |

The table clearly shows that isoelectric point of α -sericin in the first extract, or the most soluble sericin fraction, is more on the alkaline side than others, corresponding to their solubility. But the questions arise from this fact in these two points,

1. This fact may be due to the denaturation of sericin during the process of extraction.

2. Modification of isoelectric point of α -sericin slightly depends upon the concentration* of sericin sol* (See Literature).

So to verify this fact, the following experiments were carried out.

1. Influence of heating aqueous sericin sol on the modification of isoelectric point of α -sericin.

The aqueous extract at 100°C. for 10 minutes, which contained 16.94 mg. N /200 cc., of which 12.83 mg. belongs to the α -sericin at pH 4.4 (isoelectric point), was boiled for 30 minutes under the reflux condenser, and experiment was carried out in the same way as described above.

(Quantity of N is expressed in mg/200 cc.)

| Kind of Sericin Sol. | Total Nitrogen | Kind of Sericin \ pH | 3.2 | 3.4 | 3.6 | 3.8 | 4.0 | 4.2 | 4.4 | 4.6 |
|----------------------|----------------|----------------------|-------|-------|-------|-------|-------|-------|-------------|-------|
| Sericin Sol Boiled. | 16.94 | α -sericin N. | 2.24 | 3.50 | 3.99 | 3.99 | 5.11 | 5.16 | 5.46 (max) | 5.39 |
| | | β -Sericin N. | 14.70 | 13.44 | 12.95 | 12.95 | 11.83 | 11.78 | 11.48 | 11.55 |
| Control. | 16.94 | α -Sericin N. | — | — | 9.73 | 9.52 | 10.71 | 12.74 | 12.88 (max) | 12.81 |
| | | β -Sericin N. | — | — | 7.57 | 7.42 | 6.23 | 4.20 | 4.06 | 4.13 |

The difference was not found about the isoelectric point of α -sericin.

2. The modification of isoelectric point of α -sericin in soluble and insoluble sericin fractions with consideration of their concentration.

The extraction method was the same as described above, but water in each case was diminished corresponding to the times of extraction to obtain the approximately same sericin concentration of each extract.

(Quantity of N is expressed in mg/200 cc).

| Number of Extractions. | Total Nitrogen | Kind of Sericin \ pH | 3.4 | 3.6 | 3.8 | 4.0 | 4.2 | 4.4 | 4.6 | 4.8 |
|------------------------|----------------|----------------------|-------|-------|-------------|-------|-------------|------------|-------|-------|
| | | | | | | | | | | |
| 1 | 17.36 | α -Sericin N | — | — | 4.97 | 5.88 | 6.51 | 6.85 (max) | 6.02 | 5.04 |
| | | β -Sericin N | — | — | 12.3 | 11.48 | 10.85 | 10.50 | 11.34 | 12.32 |
| 2 | 15.40 | α -Sericin N | — | — | 10.50 | 10.92 | 12.74 (max) | 10.99 | 10.99 | 10.64 |
| | | β -Sericin N | — | — | 4.90 | 4.48 | 2.66 | 4.41 | 4.76 | — |
| 3* | 16.94 | α -Sericin N | 10.15 | 10.36 | 11.13 (max) | 10.85 | 10.71 | 10.57 | — | — |
| | | β -Sericin N | 6.16 | 5.95 | 5.18 | 5.46 | 5.60 | 5.74 | — | — |

* 3rd and 4th extracts were collected into one.

Through these experimental results, it is clear that isoelectric point of α -sericin in soluble sericin fraction is more on the alkaline side than that of the insoluble one, corresponding to their solubility.

(B). Modification of isoelectric point of α -sericin between the outside and the inside layer of raw cocoon.

Regarding the difference of tanning capacities and difference of the solubility of the sericin in outside and inside layer of raw cocoon, we reported in the previous paper, together with the reason for these facts.

In this part, we studied modification of isoelectric point of α -sericin which was obtained by boiling outside and inside layer of raw cocoon respectively with distilled water for only 10 minutes.

1. In the case of α -sericin in outside layer.

25 gs. of outside layer was extracted by boiling for 10 minutes in 4 l. of distilled water.

(Quantity of N is expressed in mg/200 cc).

| Total Nitrogen. | Kind of Sericin \ pH | 4.0 | 4.2 | 4.4 | 4.6 | 4.8 |
|-----------------|----------------------|------|------|------|-------------|------|
| | | | | | | |
| 17.93 | α -Sericin N | 8.93 | 9.21 | 9.91 | 10.89 (max) | 9.14 |
| | β -Sericin N | 9.00 | 8.72 | 8.72 | 7.02 | 8.79 |

2. In the case of α -sericin in inside layer.

32 gs. of inside layer was extracted by boiling for 10 minutes in 2 l. of distilled water.

(Quantity of N is expressed in mg/200 cc).

| Total Nitrogen | Kind of Sericin | pH | 3.4 | 3.6 | 3.8 | 4.0 | 4.2 | 4.4 | 4.6 |
|----------------|---------------------|----|-------|-------|------|-------|-------|----------------|-------|
| | | | | | | | | | |
| 19.67 | α -Sericin N | | 9.17 | 9.45 | 9.94 | 10.15 | 10.50 | 11.06 (max) | 10.71 |
| | β -Sericin N. | | 10.50 | 10.22 | 9.73 | 9.52 | 9.17 | 8.61 | 8.96 |

These two tables clearly show that isoelectric point of α -sericin in outside layer of raw cocoon is more alkaline than that in the inside layer, corresponding to their solubility.

3. Summary.

The work included in this paper may properly be summed up as follows;—

- (1) Isoelectric point of α -sericin in soluble sericin fraction is more alkaline than insoluble one, corresponding to their solubility.
- (2) Isoelectric point of α -sericin in outside layer of raw cocoon (soluble sericin) fraction is more alkaline than that in inside layer (insoluble sericin fraction) confirming the above result [Summary (1)].

4. Literature.

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2. Z. Hirose.: The Agr. Chem. Soc Japan, **16**, 186 43~45 (1940)
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Untersuchung über die Beziehungen von Bataten zur Alkoholproduktion.

(SS. 1107~1129)

Von Y. Takeda, M. Suematu. und M. Utikosi.

(The Institute of Research on Chemical Industry, Government-General of Taiwan,

Received 21 9 1940.)

Studien über die Flavingärung der Aceton-Butylalkoholbakterien. I

(SS. 1130~1140)

Von Izue YAMASAKI.

(Aus dem Agrikulturchemischen Institut der Kaiserlichen Kyusyu-Universität in Fukuoka; Eingegangen am 14. 10. 1940.)

Relation Between Oil Content of Fish Liver and Vitamin A Content of Liver Oil.

(pp. 1141~1150)

By Hideo HIGASHI.

(Imperial Fisheries Experimental Station, Tokyo, Japan;
Received September 28, 1940.)

The vitamin A content of fish liver oil is influenced by various factors, e. g., age, sex, spawning, fishing season, fishing ground and oil content of liver, etc. If the other factors are nearly equal, the vitamin A content of liver oil has direct connection with the oil content of liver, i. e., vitamin A content of liver oil becomes very rich when the oil content of liver decreases. This fact is observed about almost all species of fish.

The author's results are as follows.

| Species | Fishing Ground | Fishing Season | Sex | Body Length (cm) | Body Wt (g) | Liver Wt (g) | Oil Content of Liver (%) | C. L. O. U |
|------------------------------|---------------------------|-----------------|--------|------------------|-------------|--------------|--------------------------|------------|
| Katsuoanus vagans (L.). | Adjacent Sea of Palau | April, 1936 | Female | 64.0 | 6800 | 56.0 | 5.16 | 122 |
| | | | | 64.0 | 7100 | 61.0 | 3.53 | 244 |
| Sebastes flammeus J. and S. | Off the Coast of Shuogama | May 29th, 1936 | Male | 33.0 | 1056 | 23.1 | 26.8 | 204 |
| | | | | 33.0 | 1125 | 19.8 | 18.7 | 325 |
| Sebastes flammeus J. and S. | Off the Coast of Shuogama | Jan. 28th, 1937 | Male | 36.0 | 1230 | 27 | 25.4 | 170 |
| | | | | 36.0 | 1190 | 19 | 13.8 | 487 |
| | | | | 36.0 | 1070 | 21 | 9.3 | 720 |
| Sebastes flammeus J. and S. | Off the Coast of Shuogama | Feb 27th, 1937 | Male | 34.0 | 1098 | 20 | 14.5 | 242 |
| | | | | 34.0 | 1108 | 24 | 9.7 | 440 |
| Sebastes iracundus J. and S. | Off the Coast of Mito | May 6th, 1936 | Female | 57.3 | 5800 | 126 | 46.2 | 130 |
| | | | | 57.8 | 5275 | 95 | 22.7 | 568 |
| Sebastes iracundus J. and S. | Off the Coast of Choshi | May 13th, 1936 | Female | 50.0 | 3938 | 63 | 32.8 | 130 |
| | | | | 50.0 | 3638 | 53 | 21.0 | 975 |
| Sebastes iracundus J. and S. | Off the Coast of Shuogama | May 29th, 1936 | Female | 53.0 | 4500 | 93 | 16.2 | 975 |
| | | | | 53.0 | 4300 | 67 | 14.7 | 920 |

| | | | | | | | | |
|---|---------------------------|------------------|--------|------|------|------|------|------|
| <i>Sebastodes iracundus</i> J. and S. | Off the Coast of Shioyama | May 29th, 1936 | Male | 50.0 | 3700 | 45 | 16.0 | 650 |
| | | | | 50.0 | 3575 | 74 | 14.1 | 720 |
| | | | | 50.0 | 3000 | 45 | 11.3 | 1450 |
| <i>Sebastodes iracundus</i> J. and S. | Off the Coast of Shioyama | Jan. 25th, 1937 | Female | 47.0 | 2320 | 28 | 22.5 | 745 |
| | | | | 47.0 | 2610 | 30 | 13.7 | 1462 |
| <i>Sebastodes matsubarae</i> (H.). | Off the Coast of Mito | May 29th, 1936 | Female | 43.0 | 2000 | 27 | 15.8 | 568 |
| | | | | 43.0 | 2000 | 25 | 11.5 | 1140 |
| | | | | 43.0 | 2000 | 27 | 10.6 | 1210 |
| <i>Sebastodes matsubarae</i> (H.). | Off the Coast of Shioyama | Sep. 3rd, 1936 | Female | 45.0 | 2600 | 62 | 30.1 | 146 |
| | | | | 45.0 | 2600 | 41 | 22.2 | 975 |
| <i>Brama rai</i> (B.) | Off the Coast of Katsura | April 13th, 1939 | Female | 35.0 | 950 | 11.5 | 4.21 | 120 |
| | | | | 35.0 | 810 | 9.0 | 4.06 | 150 |
| | | | | 35.0 | 945 | 8.5 | 3.71 | 210 |
| <i>Seriola quinqueradiata</i> T. and S. | Off the Coast of Nagasaki | Sep. 20th, 1938 | Male | 60.0 | 4265 | 35 | 13.3 | 42 |
| | | | | 60.0 | 3855 | 30 | 6.5 | 210 |
| | | | | 60.0 | 3775 | 20 | 2.95 | 490 |
| <i>Seriola quinqueradiata</i> T. and S. | Off the Coast of Nagasaki | Sep. 20th, 1938 | Male | 63.0 | 4245 | 42 | 5.35 | 60 |
| | | | | 63.0 | 4030 | 41 | 1.92 | 336 |

In *Sebastodes flammeus* J. and S. and *Sebastodes iracundus* J. and S., oil content of liver (F) and vitamin A content of liver oil (C. L. O. U.) (A) have been determined for many individuals. According to these results the relation between F and A can be expressed as follows:

$$\log F = b - a' \log A \dots \dots \dots (I)$$

or

$$a' - F = b' \log A \dots \dots \dots (II)$$

where a , b , a' and b' are constants.

Equation (I) is proposed by the author, and equation (II) has been proposed by Schmidt-Nielsen. The former is more applicable to the case of *Sebastodes flammeus*, but the latter to the case of *Sebastodes iracundus*. In the case of *Theragra chalcogramma* (P.), either equation (I) or (II) holds good. Consequently both equations are applicable to many species of fish, but in some species equation (I) holds more true and in others equation (II).

On the Retting of Vegetable Fibre Materials. Part XIV.

(pp. 1151~1158)

By Hideo KATAGIRI and Tosio NAKAHAMA.

(Department of Agriculture, Kyoto Imperial University; Received October 14, 1940.)

In the previous papers, it was proposed by us that a useful retting bacteria revealed effective action only upon a certain kind of vegetable fibre materials.

In order to get further evidence for these specificities of retting bacteria, pectin decomposing enzymes of these bacteria were compared.

All the useful retting bacteria including one species of bacteria for ramie, four species for hemp, three species for flax, two species for kenaf and one species for jute fibre materials, were found to reveal very much the same activity of pectase with which Ca-tartrate was produced from methyl-d-Ca-tartrate.

The action of pectinase with which lemon pectin was decomposed, was found to be different among the species of retting bacteria, i. e. *B. linum* for flax, *Achromobacter venosum* for flax, *Microc. cannabis* for hemp, and *Listerella hibiscus liquefaciens* for kenaf attacked pectin very remarkably, while *B. subtilis* for ramie, *B. cannabis* for hemp and *Kurthia cannabis liquefaciens* for hemp attacked slightly on pectin.

Therefore, any parallel relation was not found to exist between the kinds of fibre materials and the activity of pectase or pectinase of the bacteria.

However, very remarkable specificities were pointed out between the activity of bacterial protopectinases and the kinds of protopectin prepared from various kinds of fibre materials.

These specificities of bacterial protopectinases were found to be very much the same as those of the bacterial rettings of vegetable fibre materials.

Biochemistry of *Bakanae* Fungus. Part VII.

The Cultural Condition for Producing Gibberellin
or Fusaric Acid. II.

(pp. 1157~1158)

By T. YABUTA, Y. SUMIKI, E. KATAYAMA and H. MOTOYAMA.

(Agricultural Chemical Laboratory, Tokyo Imperial University,

Received October 25, 1940.)

ABSTRACTS**from****TRANSACTIONS published in JAPANESE**

(Pages refer to the Japanese originals of this volume unless otherwise noted.)

**Studies on the Chemical Constituents of
"Inekoji." Part VII.**

The Red Pigment, Ustilaginoidin (IV).

(pp. 1159~1161)

By Teijiro YABUTA, Yusuke SUMIKI and Kimiko ANNO.

(Tokyo Imperial University, Received November 20, 1940)

On the Catalase in Juice of Fruits, Roots or Stems.

(pp. 1162~1166)

By Hisao MATUI.

(The Governmental Institute of Brewing, Takinogawa, Tokyo,

Received September 20, 1940.)

The catalase action of juice of fruits (about 30 sorts) and of tubers, bulbs, roots or stems of various vegetables was examined. The results may be summarized as follows:

1. The concentration of hydrogen ion in the medium in which the reaction is occurring influences the catalase action of fruit juice. The optimum concentration for the catalase action lies between pH 7 and 8 with some exceptions—5.8 (tomato), 6.0 (apple) and 8.6 (persimmon).

2. The catalase action of fruit juice is different in strength according to families of plants. Generally orange and grape juices are feeble and that of melons strong.

3. In general, hydrogen ion concentration of fruit juice has a great influence upon the catalase content. If pH value of juice is small, the catalase action is weak and according as pH value approaches 7 the catalase action becomes gradually strong, but a few exceptions are recognized.

4. Juice of tubers, bulbs, roots or stems of vegetables contains a larger quantity of catalase than juice of ordinary fruits.

On the Oxidative Substance Appearing in Juice of Salted Vegetables.

(pp. 1167~1168)

By Hisao MATSU.

(The Governmental Institute of Brewing, Takinogawa, Tokyo;

Received September 20, 1940.)

The existence of nitrite has been demonstrated in juice of salted vegetables (garden radish, turnip, cabbage and various greens). When the vegetable is salted, nitrite is detected in the juice by Griess' test in a few days, and this may be perhaps produced by bacteria (lactic acid bacterium, etc.) from nitrate which is contained in vegetable juice. The amount of nitrite often reaches 230.5 mg as N_2O_5 in 100 cc of salted juice. But it gradually disappears as nitrous anhydride is formed in acidic condition.

On the Cellulose Analysis and Bleaching Methods of Cellulose Materials. Part IV.

Application of the Modified New Method of Cellulose Estimation on Various Plant Analyses.

(pp. 1169~1175)

By Sin-iti HONDA.

(Kyoto Imperial University; Received October 19, 1940)

In the previous papers, the present author proposed the new method, modification of Jenkins' original method. With the view to proving the general usefulness of the modified method the present author applied as an example this method for the cellulose analysis of several plant materials. The results are tabulated in Table I

Table I. Comparison of plant cellulose contents by different analytical methods with various materials. (Oven dry state.)

| Compositions, | Phase of chlorination, | Liquid Phase (Bleaching powder solution) | | Gaseous Phase |
|--|------------------------------------|---|-----------------|---------------------------------------|
| | Analytical method, | Previous Method, | Modified Method | Cross and Bevan's Modified Method, |
| Hitujigusa (<i>Poa glumaris</i> , Tri.) | | | | |
| Total cellulose (%) | | 47.52±0.46 | 44.77±0.03 | 44.10±0.46 |
| α -cellulose (ash-free) (%) | | 34.45±0.89 | 33.10±0.09 | 32.55±0.88 |
| In total cellulose { | α -cellulose (ash-free) (%) | 72.51±1.80 | 73.92±0.26 | 73.77±1.16 |
| | α -cellulose (%) ash (%) | 0.99 | 1.05 | — |
| | β -cellulose (%) | 7.75 | 25.03 | 25.29 |
| | γ -cellulose (%) | 19.75 | | |
| Number of chlorinations, | | 2N, 2A | 3A | ? |

Goyō no matu (*Pinus parviflora*)

| | | | |
|----------------------------|----------------------------|------------|------------|
| Total cellulose (%) | 56.03±0.06 | 55.29±0.43 | 52.55±0.09 |
| α-cellulose (ash-free) (%) | 39.12±0.08 | 39.02±0.28 | 34.37±0.41 |
| In total cellulose | α-cellulose (ash-free) (%) | 69.82±0.09 | 70.42±0.12 |
| | α-cellulose ash (%) | 0.23 | 0.33 |
| | β-cellulose (%) | 30.02 | 29.25 |
| | γ-cellulose (%) | | |
| Number of chlorinations. | 2N, 4A | 4A | ? |

Chosen Gōyo no matu (*Pinus koraiensis*, Sieb et Zucc.)

| | | | |
|----------------------------|----------------------------|------------|------------|
| Total cellulose (%) | 52.44±0.12 | 51.56±0.36 | 49.92±0.06 |
| α-cellulose (ash-free) (%) | 37.97±0.47 | 37.10±0.52 | 30.03 |
| In total cellulose | α-cellulose (ash-free) (%) | 72.40±0.35 | 71.96±0.76 |
| | α-cellulose ash (%) | 0.04 | 0.34 |
| | β-cellulose (%) | 2.22 | 27.70 |
| | γ-cellulose (%) | 25.34 | |
| Number of chlorinations. | 2N, 5A | 5A | ? |

Hosoba isotutaji (*Ledum palustre* L. var. *vulgare* Ledeb.)

| | | | |
|----------------------------|----------------------------|------------|------------|
| Total cellulose (%) | 35.32±0.48 | 35.47±0.10 | |
| α-cellulose (ash-free) (%) | 22.73±0.58 | 21.89±0.40 | |
| In total cellulose | α-cellulose (ash-free) (%) | 64.39±2.26 | 61.70±1.25 |
| | α-cellulose ash (%) | 5.26 | 1.90 |
| | β-cellulose (%) | 30.35 | 37.40 |
| | γ-cellulose (%) | | |
| Number of chlorinations. | 2N, 6A | 6A | |

Sirakanba (*Betula japonica* Sieb or *B. latifolia* Kom.)

(1) Sapwood

| | | | |
|----------------------------|----------------------------|------------|------------|
| Total cellulose (%) | 57.93±0.88 | 58.06±0.37 | |
| α-cellulose (ash-free) (%) | 42.13±0.65 | 41.48±0.18 | |
| In total cellulose | α-cellulose (ash-free) (%) | 72.65±0.21 | 71.44±0.17 |
| | α-cellulose ash (%) | 0.27 | 0.26 |
| | β-cellulose (%) | 15.01 | 18.72 |
| | γ-cellulose (%) | 12.07 | 9.78 |
| Number of chlorinations. | 2N, 4A. | 4A | |

(2) Heartwood

| | | | |
|----------------------------|----------------------------|------------|------------|
| Total cellulose (%) | 53.68±1.18 | 57.06±0.25 | |
| α-cellulose (ash-free) (%) | 37.47±0.62 | 38.56±0.16 | |
| In total cellulose | α-cellulose (ash-free) (%) | 69.83±0.37 | 67.59±0.19 |
| | α-cellulose ash (%) | 0.30 | 0.32 |
| | β-cellulose (%) | 29.87 | 32.09 |
| | γ-cellulose (%) | | |
| Number of chlorinations. | 2N, 3A | 3A | |

It will be seen that the total cellulose contents were always higher in the modified method than given in the original paper. However, the mean difference is about 1.5 %, and thus the modified method may be quite sufficient for use in the pulp and paper industries.

Moreover, with regard to the α -cellulose contents shown in Table I, the results of analysis with the modified method show good agreement with Jenkins' original method.

The experimental results by Cross & Bevan's chlorination method obtained in the present author's laboratory were also tabulated in Table I, for comparison.

Thus it was seen that Jenkins' original method may be used instead of Cross & Bevan's chlorination method. Moreover, the modified method proposed by the present author may be recommended as an improved and simplified method in place of Jenkins' original method.

(Prof. Sakata's Laboratory, The Institute of Chemical Research, Kyoto Teikoku Daigaku.)

On the Denaturation of Sericin. Part 3.

Some References to the Denaturation of $\alpha_{3,3}$ -Sericin
Powder with $\alpha_{4,4}$ -Sericin Powder.

(pp. 1176~1180)

By Zirō HIROSE.

1. INTRODUCTION.

In the previous paper⁽¹⁾, we studied isoelectric point of α -sericin and found isoelectric point of α -sericin in soluble sericin fraction is more alkaline than that of insoluble one, corresponding to their solubility.

In this paper we studied some references of denaturation of $\alpha_{3,3}$ -sericin (obtained by Ito and Komori's method⁽²⁾) powder with $\alpha_{4,4}$ -sericin (obtained soluble sericin fraction⁽¹⁾, powder stoichiologically. But in this and further reports, designation of α -sericin, in details, was followed by the next example.

A. α -sericin, being precipitated at pH 4.4..... $\alpha_{4,4}$ -sericin and so on.

B. α -sericin, being obtained as insoluble part when original α -sericin was boiled with distilled water for definite time,..... $\alpha_{1,1}$ -sericin. If we wish to show their isoelectric point,..... $\alpha_{4,4}$ -sericin, and so on.

2. EXPERIMENTAL.

(1) Preparation and isolation of α -sericins.

(A) $\alpha_{3,3}$ -sericin⁽³⁾.

200 gs. of raw cocoons, being freed from chrysalid, extracted by boiling (110°C) with 6 l. of distilled water for 30 minutes. Extraction was repeated twice. All the extracts were collected, and to this sericin sol added acetate mixture of

pH 3.8 (final conc. ... 0.02 M). Precipitate thus formed was brought to the powdered state by means of alcohol and ether.

Yield, ... 27.2 gs. N %, ... 16.78 %.

(B) α_{44} -sericin⁽¹⁾

388 gs. of raw cocoon layers were extracted by boiling for only 10 minutes with 10 l. of distilled water, and precipitate at pH 4.4 was brought to the powdered state by means of alcohol and ether.

Yield, ... 12.7 gs. N %, ... 17.28 %.

(2) Treatment of α_{38} -sericin with boiling water and isolation of α_1 -sericin.

20 gs. of powdered α_{38} -sericin was treated with 5 l. of boiling water for 30 minutes. Insoluble part of α_{38} -sericin was collected on the glass filter and brought to the powdered state by means of alcohol and ether.

Yield, ... 10.6 gs. N %, ... 17.13 %.

(3) Treatment of α_{38} -, α_{44} -, and α_1 -sericin with tannic acid.

0.2 gs. of powdered sericins were treated with tannic acid of 10.00 gs/l. concentration, kept at 25°C for 3 hours.

| Kind of Sericin | α_{44} -sericin | α_{38} -sericin | α_1 -sericin |
|--------------------------------|------------------------|------------------------|---------------------|
| Tannin adsorbed in percentage. | 9.92 | 8.24 | 10.16 |

Table clearly shows that adsorption of α_1 -sericin with tannic acid is very similar to that of α_{44} -sericin, and not to α_{38} -sericin.

(4) Determination of isoelectric point of α_{44} -, α_1 -, and α_{38} -sericin by dye technic.

Leob⁽²⁾ showed that acid dye combined with collagen on the acid side of its isoelectric point and basic dye combined with collagen on the alkaline side of its isoelectric point. We used this principle to measure the isoelectric point of sericin. The procedure was as follows;—

0.3 gs. of dried sericin was kept for 10 hours in 50 cc. of acetate mixture of given pH value (0.02 m) and then for 8 hours in another 50 cc. of buffer containing dye (final dye conc. was equal to 0.005 %). Uncombined dye was deter-

| Kind of Sericin | Dyestuff | pH | 3.4 | 3.6 | 3.8 | 4.0 | 4.2 | 4.4 | 4.6 | 4.8 |
|------------------------|---------------------------|----|------|------|------|------|------|------|------|------|
| α_{44} -Sericin | Orange G, adsorbed in (%) | | — | — | — | 0.91 | 0.84 | 0.76 | 0.64 | 0.57 |
| | Ditto to methylene blue | | — | — | — | 0.91 | 0.84 | 0.76 | 0.64 | 0.57 |
| α_1 -Sericin | Orange G, adsorbed in (%) | | 1.20 | 1.16 | 1.04 | 0.96 | 0.81 | 0.74 | 0.58 | — |
| | Ditto to methylene blue | | 0.37 | 0.44 | 0.53 | 0.71 | 0.82 | 0.87 | 0.88 | — |
| α_{38} -Sericin | Orange G, adsorbed in (%) | | 0.92 | 0.86 | 0.82 | 0.77 | — | 0.71 | 0.59 | — |
| | Ditto to methylene blue | | 0.69 | 0.78 | 0.82 | 0.88 | — | 0.98 | 1.04 | — |

mined by colorimetry. Dyestuffs used were Orange G (as acid dye) and methylene blue (as basic dye). Experimental results are shown in the following Table and figs. (see figs. 1, 2 and 3).

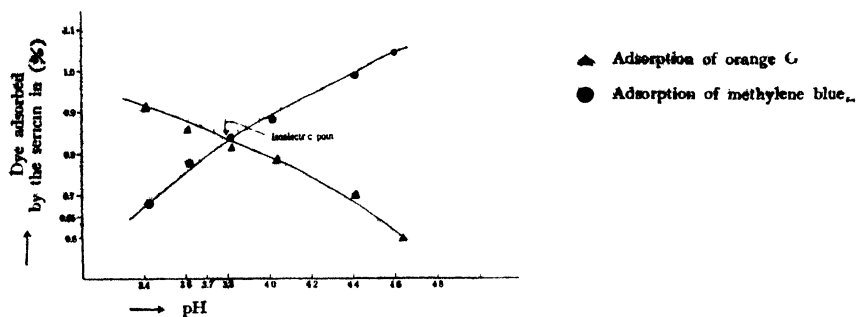


Fig 1 Determination of Isoelectric Point of $\alpha_1\beta$ -Serum by the Dye Technic

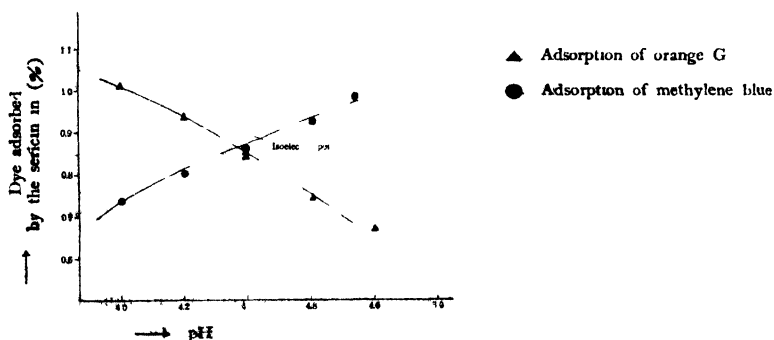


Fig II Determination of Isoelectric Point of $\alpha_1\gamma$ -Serum by the Dye technic.

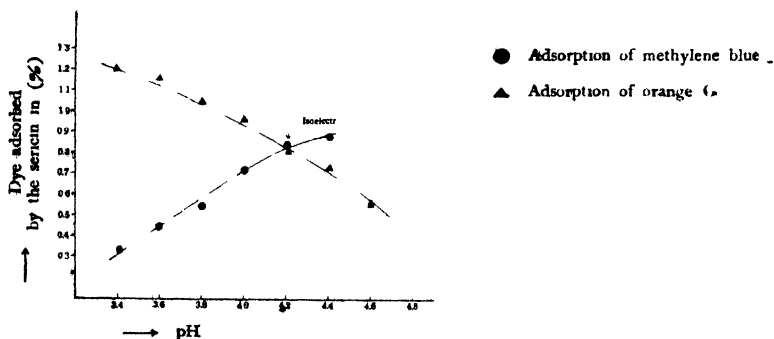


Fig III Determination of Isoelectric Point α_1 serum by the Dye Technic

(5) Combination of $\alpha_{4,4}$, α_1 - and $\alpha_{3,8}$ -sericin with iodine.

0.2gs. of dried sericins were treated with 50cc. of 0.079 *N.* iodine, kept at 25 for 3 hours.

| Kind of Sericin | $\alpha_{4,4}$ -Sericin | $\alpha_{3,8}$ -Sericin | α_1 -Sericin |
|--|-------------------------|-------------------------|---------------------|
| Iodine combined per gs. of sericin in gs | 0.088 | 0.077 | 0.089 |

3. SUMMARY.

The work included in this paper may properly be summed up as follows,

(1) $\alpha_{4,4}$ -sericin and α_1 -sericin takes up more acid dyes and tannic acid than $\alpha_{3,8}$ -sericin, while, on the contrary, $\alpha_{3,8}$ -sericin takes up more basic dyes than $\alpha_{4,4}$, and α_1 -sericin.

(2) $\alpha_{4,4}$ -sericin and α_1 -sericin combines more iodine than $\alpha_{3,8}$ -sericin, indicating $\alpha_{4,4}$, and α_1 -sericin has more aromatic amino acid⁽⁶⁾ and tryptophane than $\alpha_{3,8}$ -sericin.

A. With regard to the isoelectric point of $\alpha_{3,8}$ -sericin, its isoelectric point is 3.7~3.8, being agreed with the announcement already made by Dr. Ito⁽²⁾.

B. With regard to the isoelectric point of $\alpha_{4,4}$ -sericin its isoelectric point is 4.3~4.4, being agreed with my report already made in the previous paper⁽⁷⁾.

C. The isoelectric point of α_1 -sericin is near 4.2. This fact clearly shows, when α -sericin is treated with hot water, insoluble part of α -sericin, or α_1 -sericin, is more on the alkaline side than original one.

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Studies on the Vitamins of Fish Livers. (Part II.)

Relation Between Age of Fish and Vitamin A

Content of Liver Oil.

(pp. 1181~1188)

By Hideo HIGASHI

(Imperial Fisheries Experimental Station, Tokyo, Japan;

Received November 15, 1940.)

I have observed that in several species of fish, if all conditions except age (or size) are nearly equal, liver oils of older fish are richer in vitamin A than those of younger fish. I believe that the older fish consumes less vitamin A for unit body weight than younger fish. This is the reason why the liver oils of

older fish are richer in vitamin A than those of younger fish. When all conditions, other than age, are nearly equal, the amount of vitamin A consumed per unit of body weight would be proportional to the velocity of growth. So the relation between age of fish and vitamin A content of liver oil can be expressed by a curve related to the growth curve of fish.

According to this assumption, it is easily presumed that the liver oils of very old fish in each species are extraordinarily rich in vitamin A.

Results which I have obtained are as follows:—

| No. | Species | Fishing Season | Fishing Ground | Sex | Body Length cm. | Body Wt. g. | Liver Wt. Body Wt. (%) | Oil Content of Liver (%) | C. L. O. U. |
|-----|---|-----------------|----------------------------|--------|-----------------|-------------|------------------------|--------------------------|-------------|
| 1 | <i>Cynopsetta dubia</i> S. | June 28th, 1933 | Bering Sea | Female | 70.5 | 4900 | 3.57 | 13.2 | 143 |
| 2 | <i>Gadus macrocephalus</i> T. | June 25th 1933 | Bering Sea | Female | 92.0 | 12600 | 3.05 | 6.98 | 500 |
| 3 | <i>Sebastes flammeus</i> J. and S. | Apr. 10th, 1936 | Off the Coast of Shioyama | Male | 45.0 | 2250 | 1.24 | 8.30 | 2240 |
| 4 | <i>Sebastes iracundus</i> J. and S. | May 6th, 1936 | Off the Coast of Mito | Female | 63.0 | 6200 | 1.66 | 15.3 | 2880 |
| 5 | <i>Etelis carbunculus</i> C. and V. | Dec. 20th, 1937 | Off the Coast of Kagoshima | Male | 65.0 | 6500 | 0.37 | 5.70 | 600 |
| 6 | <i>Papacaecio caeruleus</i> (K.). | Dec. 20th, 1937 | Off the Coast of Kagoshima | Male | 39.5 | 1900 | 0.36 | 46.7 | 350 |
| 7 | <i>Ocyrius japonicus</i> D. | Dec. 20th, 1937 | Off the Coast of Kagoshima | Female | 64.0 | 7600 | 0.78 | 2.44 | 900 |
| 8 | <i>Xiphias gladius</i> L. | Apr. 14th, 1938 | Adjacent Sea of Hachijo | Male | 178.0 | 28970 | 1.17 | 5.88 | 450 |
| 9 | <i>Pristipomoides sieboldi</i> (B.). | Dec. 20th, 1939 | Off the Coast of Kagoshima | Female | 60.0 | 4530 | 0.64 | 5.26 | 1260 |
| 10 | <i>Neothunus macropterus</i> (T. and S.). | Feb. 1st, 1940 | Adjacent Sea of Parao | Male | 125.0 | 46500 | 0.58 | 2.32 | 840 |

Dietary Studies on the Increase of Utilizing Value of Northern Farm Animals. I.

Hair Growth and Feed.

(pp. 1189~1199)

By E. TAKAHASHI and K. SHIRAHAMA.

(Department of Agriculture, Hokkaido Imperial University,

Received November 25, 1940.)

Various kinds of feed were analysed for their cystine contents¹ and a few basic experiments on the relation of the hair growth and feed were carried out on albino rats.

Studies on the Lipids of Salmon Eggs.

(1) On the Acetone Soluble Fraction.

(pp. 1200~1206)

By Kimiko ANNO.

(Agricultural Chemical Laboratory, Tokyo Imperial University;

Received November 25, 1940.)

Salmon eggs, *Oncorhynchus Gorbushola*, were extracted with methyl alcohol, petroleum ether and ether. The lipids obtained were separated into phosphatides and fatty oil with acetone.

The fatty oil on saponification gave fatty acids and unsaponifiable matter.

The fatty acids were separated into about 15 per cent of solid and 85 per cent of liquid acids. The solid acid mainly consisted of palmitic acid. The liquid acid contained about half oleic acid and a considerable amount of clupanodonic acid. These acids were isolated and identified. Arachidonic acid probably was present also.

The unsaponifiable matter consisted chiefly of cholesterol.

Sterilizing Action of Acids and Phenols.

(pp. 1207~1224)

By Sogo TETSUMOTO.

(Government Institute for Infectious Diseases, Tokyo Imperial University,

Received November 4, 1940)

13th Report. Relation between the Chemical Constitution of Phenols and Aromatic Acids and Physiology of Bacteria.







Concerning the relation between the chemical constitution of fatty acids such as normal, iso, cis, trans, d, l, i, meso, and the physiology of bacteria, I have previously reported.







Also concerning the relation between the chemical constitution of phenols such as pyrocatechin (*o*), resorcin (*m*), hydroquinon (*p*), and pyrogallie acid (*o*), phloroglucin (*m*), and the sterilizing action of promoting action, it is reported in my previous paper. Among aromatic acids there are many isomers having different chemical constitutions.









To find what relation exists between the various isomers having different chemical constitutions and the physiology of bacteria, I performed the next experiment. Reagents used are shown in the following table.

§ 1. Reagents.

Table I. Reagents and constitution formulae.

| Phenols | Isomer | Chemical constitution | M P | B P |
|---|----------|---|-------|----------|
| Cresol $C_6H_4 \cdot CH_3 \cdot OH$ M W 108.064 | <i>o</i> |  | 30° | 191° |
| | <i>m</i> |  | 44° | 203° |
| | <i>p</i> |  | 36° | 138° |
| Chlorophenol $C_6H_4 \cdot Cl \cdot OH$ M W 128.530 | <i>o</i> |  | 7° | 175~176° |
| | <i>m</i> |  | 28.5° | 212° |
| | <i>p</i> |  | 37° | 217° |

| | | | | |
|--|----------|---|--------|----------|
| Bromophenol $C_6H_4 \cdot Br \cdot OH$ M W 173 030 | <i>o</i> |  | | 195~198° |
| | <i>m</i> |  | 32~33° | 236° |
| | <i>p</i> |  | 64° | 238° |
| Nitrophenol $C_6H_4 \cdot NO_2 \cdot OH$ M W 139 078 | <i>o</i> |  | 45° | 214° |
| | <i>m</i> |  | 96° | 194° |
| | <i>p</i> |  | 114° | |

| Aromatic acids | Isomer | Chemical constitution | M P |
|---|-------------|---|--------------------------|
| Benzoic acid $C_6H_5 \cdot CO_2H$ M W. 122 048 | |  | 121° |
| Salicylic acid $C_6H_4 \cdot OH \cdot CO_2H$ M W 138 048 | <i>o</i> |  | 156°~157° |
| Meta oxybenzoic acid | <i>m</i> |  | 188° |
| Para oxybenzoic acid | <i>p</i> |  | 213° |
| Phthalic acid $C_6H_4 \begin{cases} CO_2H \\ CO_2H \end{cases}$ M W 166 048 | |  | |
| | <i>nor</i> |  | 196°~199° |
| | <i>iso</i> |  | 332°~335° |
| | <i>tele</i> |  | over 300° sublimes |

§ 2. Relation between the chemical constitutions of phenols and aromatic acids and the sterilizing action at the same concentration.

To find what relation exists between the chemical constitutions of phenols and aromatic acids and the sterilizing action on bacteria, I performed this experi-

ment. Concentration of each reagent was made $N/1000$, only phthalic acids were made $N/100000$, because they are hardly soluble in water. Results obtained are as shown in the following tables.

Table 2 Relation between the chemical constitution of phenols and the strength of sterilizing action.

| Phenols | Isomer | pH | Surviving period | | | |
|------------------------|--------|------|------------------|-----------------|------------------|-----------------|
| | | | Staph pyog | P vulgar | B typhosus | V cholerae |
| Cresol | o | 5.36 | $6^d + 7^d -$ | $4^d \pm 5^d -$ | $5^d + 6^d -$ | $6^h \pm 9^h -$ |
| | m | " | $7 + 8 -$ | $5 + 6 -$ | $7 \pm 8 -$ | $9 + 12 -$ |
| | p | 5.57 | $5 + 6 -$ | $5 + 6 -$ | $3 + 4 -$ | $3 + 6 -$ |
| Cl phenol | o | 5.73 | $4 + 5 -$ | $2 + 3 -$ | $3 + 4 -$ | $60^m + 90^m -$ |
| | m | 5.76 | $3 \pm 4 -$ | $24^h + 36^l -$ | $2 + 3 -$ | $45 + 60 -$ |
| | p | " | $2 + 3 -$ | $18 + 24 -$ | $36^h \pm 2 \pm$ | $30 + 45 -$ |
| Br phenol | o | 5.76 | $3 \pm 4 -$ | $24 + 36 -$ | $2^d + 3^d -$ | $45 \pm 60 -$ |
| | m | 5.80 | $2 + 3 -$ | $18 + 24 -$ | $24^h + 36^h -$ | $30 + 45 -$ |
| | p | " | $36^l + 2 -$ | $12 + 18 -$ | $18 + 24 -$ | $20 + 30 -$ |
| NO ₂ phenol | o | 5.76 | $2^d + 3^d -$ | $24 \pm 36 -$ | $2^d \pm 3^d -$ | $30 \pm 45 -$ |
| | m | | $24^h + 36^h -$ | $12 + 18 -$ | $18^h + 24^h -$ | $20 + 30 -$ |
| | p | 5.78 | $18 + 24 -$ | $9 + 12 -$ | $12 + 18 -$ | $15 + 20 -$ |
| Control | | | $8^d \pm$ | $5^d \pm$ | $6^d \pm$ | $18^h \pm$ |

Table 3. Relation between the chemical constitution of aromatic acids and the strength of the sterilizing action.
($N/1000$, only phthalic acids $N/100000$)

| Acid | Isomer | pH | Surviving period | | | |
|-----------|--------|------|------------------|-----------------|-----------------|-------------------|
| | | | Staph pyog | P vulgar | B typhos | V choier |
| Benzoic | | 3.58 | $12^h + 24^h -$ | $6^h \pm 9^h -$ | $9^h + 12^h -$ | $20^m + 30^m -$ |
| Salicylic | o | 3.08 | $90^m + 2^h -$ | $45^m + 60^m -$ | $60^m + 90^m -$ | $2.5^m \pm 5^m -$ |
| m-OH-benz | m | 3.68 | $9^h + 12 -$ | $6^h \pm 9^h -$ | $6^h + 9^h -$ | $20^m \pm 30^m -$ |
| p OH-benz | p | " | $9 \pm 12 -$ | $3 + 6 -$ | $6 \pm 9 -$ | $15 + 20 -$ |
| Phthalic | nor | 4.34 | $2^d + 3^d -$ | $36^h + 2^d -$ | $2^d \pm 3^d -$ | $90^m \pm 2^d -$ |
| | iso | 4.83 | $3 + 4 -$ | $2^d \pm 3^d -$ | $2 + 3 -$ | $2^h + 3^h -$ |
| | tele | 4.13 | $4 + 5 -$ | $3 \pm 4 -$ | $3 + 4 -$ | $3 + 6 -$ |
| Control | | | $8^d \pm$ | $5^d \pm$ | $6^d \pm$ | $18^h \pm$ |

From the results obtained I found the following facts. The sterilizing action of *p* isomers is the strongest of all the phenols. In cresols the degree of the strength of the sterilizing action is as follows:— $m < o < p$.

Among halogen phenols and NO_2 phenols the strength of the sterilizing action is as follows:— $o < m < p$.

The sterilizing action of phenols has no relation to pH of each phenol. The sterilizing action of O-OH-benzoic acid is the strongest among OH benzoic acid isomers. The order of the strength of OH benzoic acid isomers is as follows:— $m < p < o$.

The chief cause of difference of the sterilizing action is based on pH and partly on each position of OH group combined at benzene ring. Among phthalic acid isomers the order of the strength of the sterilizing action is as follows:—

tele < iso < normal.

And pH of each isomer is as follows:— tele < normal < iso.

Accordingly there seems to exist no relation between the sterilizing action of each isomer and pH.

§ 3. The action of salts and anions of phenol isomers and aromatic acid isomers on the physiology of bacteria.

To examine how the anions of phenol isomers and aromatic acid isomers act on microorganisms, I made aqueous solution of Na, Ca, and NH_4 salts, each having the same anions as each phenol isomer or aromatic acid isomer respectively, and performed this experiment. Except phthalic acid salts the concentration of salts was made $N/1000$. Concentration of phthalic acid salts was made $N/100000$.

Table 4. The action of neutral salts of phenols and aromatic acids.

I. Na salts of phenols.

| Na — | Isomer | Surviving period | | | |
|--------------|----------|-----------------------------------|-------------------------------------|-----------------------------------|------------------------------------|
| | | Staph. pyogen | Prot. vulgar. | Bac. typhos. | Vib. cholera. |
| Cresolate | <i>o</i> | 7 ^d — 9 ^d | 5 ^d — 6 ^d | 6 ^d — 7 ^d | 9 ^h + 12 ^h — |
| | <i>m</i> | 9 — 11 | 6 — 7 | 8 — 10 | 24 + 36 — |
| | <i>p</i> | 6 — 7 | 4 — 5 | 5 — 6 | 6 + 9 — |
| Cl-phenolate | <i>o</i> | 6 ^d ± 7 ^d — | 4 + 5 — | 5 ^d ± 6 ^d — | 6 + 9 — |
| | <i>m</i> | 4 + 5 — | 3 + 4 — | 3 + 4 — | 5 + 8 — |
| | <i>p</i> | 3 + 4 — | 2 + 3 — | 2 + 3 — | 3 + 5 — |
| Br-phenolate | <i>o</i> | 5 ± 6 — | 2 + 3 — | 4 + 5 — | 3 + 6 — |
| | <i>m</i> | 3 ± 4 — | 36 ^h + 2 — | 2 + 3 — | 2 + 3 — |
| | <i>p</i> | 2 + 3 — | 24 ^h + 36 ^h — | 2 ± 3 — | 90 ^m + 2 — |

| | | | | | |
|----------------------------|----------|------------------|-------------------------------------|-------------------------------------|-------------------------------------|
| NO ₂ -phenolate | <i>o</i> | 4 + 5 - | 2 ^d ± 3 ^d - | 3 + 4 - | 2 ^h + 3 - |
| | <i>m</i> | 3 ± 4 - | 36 ^h + 2 - | 2 + 3 - | 90 ^m + 2 - |
| | <i>p</i> | 2 + 3 - | 24 ^h ± 36 ^h - | 24 ^h + 36 ^h - | 60 ^m + 90 ^m - |
| Control | | 8 ^d ± | 5 ^d ± | 6 ^d ± | 18 ^h ± |

II. Na salts of aromatic acids.

| Na— | Isomer | Surviving period | | | |
|----------------------|-------------|-------------------------------------|----------------------------------|-----------------------------------|-------------------------------------|
| | | Staph pyogen | Prot vulgar | Bac typhos | Vib cholér |
| Benzoate | | 15 ^d - 18 ^d - | 8 ^d - 10 ^d | 10 ^d - 13 ^d | 18 ^h + 24 ^h - |
| Salicylate | <i>o</i> | 4 - 5 | 2 - 3 | 3 - 4 | 30 ^m + 45 ^m - |
| <i>m</i> -o-benzoate | <i>m</i> | 10 - 13 | 6 - 8 | 8 - 10 | 12 ^h + 24 ^h - |
| <i>p</i> -o-benzoate | <i>p</i> | 8 - 10 | 5 - 7 | 6 - 8 | 9 + 12 - |
| Phthalate | <i>nor</i> | 15 - 20 | 8 - 10 | 12 - 15 | 9 ± 12 - |
| | <i>iso</i> | 20 - 25 | 10 - 13 | 15 - 18 | 12 + 18 - |
| | <i>tele</i> | 25 - 30 | 17 - 20 | 20 - 25 | 18 + 24 - |
| Control | | 8 ^d ± | 5 ^d ± | 6 ^d ± | 18 ^h ± |

Since the results of Na, Ca, and NH₄ salts were nearly the same, I have described the results of Na salts only.

From the results above noted, we can deduce these facts:

- (1) The order of preventing power for the survival of bacteria is as follows.
 - a. Salts of cresol isomers, $m < o < p$
 - b. Salts of halogen phenol and NO₂ phenol isomers, $o < m < p$.
 - c. Salts of OH substituted benzoic acid isomers, $m < p < o$
 - d. Salts of phthalic acid isomers, $tele < iso < normal$.

From these facts we can deduce the following:

- (2) Among cresol isomers, only anion of para isomer has the preventing power for bacteria, but anions of *o* and *m* have no such power.
- (3) The strength of preventing power of anions of halogen phenol and NO₂ phenol for the survival of bacteria is as follows: $o < m < p$.
- (4) Among anions of benzoic acid and its OH substituted acids, only anions of O-OH-benzoic acid (salicylic acid) have the preventing action on the survival of bacteria, but other anions such as *m* or *p* have none.
- (5) Anions of phthalic acid isomers have no preventing action.

§ 4. Sterilizing action of phenols and aromatic acids isomers at the same pH solution.

To find the relation between the strength of sterilizing power of *o*, *m*, and *p* isomers of phenols or aromatic acids and the chemical constitution of each reagent,

I made an aqueous solution of each reagent, making the aqueous solution of pH 5.45 with cresols and that of pH 5.80 with halogen phenols and NO₂ phenols at

Table 5. Effects of *o*, *m*, and *p* phenol and aromatic acid isomers on the physiology of bacteria at the same pH.

I. Phenols.

| Phenols | Isomer | Concent. | pH | Surviving period | | | |
|-------------------------|----------|----------|------|-----------------------------------|-----------------------------------|-------------------------------------|------------------------------------|
| | | | | Staph. pyogen. | Prot. vulgar. | Bac. typhos | Vib. cholera |
| Cresol | <i>o</i> | N/2000 | 5.45 | 6 ^d + 7 ^d - | 5 ^d + 6 ^d - | 6 ^d + 7 ^d - | 6 ^h + 9 ^h - |
| | <i>m</i> | " | " | 8 + 9 - | 6 + 7 - | 7 + 8 - | 12 ± 18 - |
| | <i>p</i> | N/1000 | " | 5 + 6 - | 3 ± 4 - | 3 + 4 - | 3 + 6 - |
| Cl-phenol | <i>o</i> | N/2000 | 5.80 | 5 + 6 - | 3 + 4 - | 4 + 5 - | 90 ^m + 2 ^h - |
| | <i>m</i> | N/1500 | " | 3 + 4 - | 36 ^h ± 2 - | 2 + 3 - | 60 + 90 ^m - |
| | <i>p</i> | " | " | 2 + 3 - | 24 + 36 ^h - | 36 ^h + 2 ^d - | 30 + 45 - |
| Br-phenol | <i>o</i> | N/1500 | " | 3 + 4 - | 36 ± 2 ^d - | 2 ^d + 3 - | 45 + 60 - |
| | <i>m</i> | N/1000 | " | 2 + 3 - | 18 + 24 ^h - | 24 ^h + 36 ^h - | 30 + 45 - |
| | <i>p</i> | " | " | 36 ^h + 2 - | 12 + 18 - | 18 + 24 - | 20 + 30 - |
| NO ₂ -phenol | <i>o</i> | N/1500 | " | 3 ^d ± 4 - | 24 + 36 - | 2 ^d + 3 ^d - | 30 + 45 - |
| | <i>m</i> | " | " | 36 ^h + 2 - | 12 + 18 - | 24 ^h + 36 ^h - | 20 + 30 - |
| | <i>p</i> | N/1000 | " | 18 + 24 ^h - | 9 + 12 - | 12 + 18 - | 15 + 20 - |
| Control | | | | 8 ^d ± | 5 ^d ± | 6 ^d ± | 18 ^h ± |

II. Aromatic acids.

| Acid | Isomer | Concent | pH | Surviving period | | | |
|---------------------|-------------|----------|------|-------------------------------------|------------------------------------|-------------------------------------|-------------------------------------|
| | | | | Staph. pyogen | Prot. vulgar. | Bac. typhos. | Vib cholera |
| Benzoic | | N/1100 | 3.68 | 18 ^h ± 24 ^h - | 6 ^h + 9 ^h - | 12 ^h + 18 ^h - | 20 ^m + 30 ^m - |
| Salicylic | <i>o</i> | N/6000 | " | 3 + 6 - | 90 ^m + 2 ^h - | 2 + 3 - | 10 + 15 - |
| <i>m</i> -o-benzoic | <i>m</i> | N/1000 | " | 9 + 12 - | 6 ± 9 - | 6 + 9 - | 20 ± 30 - |
| <i>p</i> -o-benzoic | <i>p</i> | " | " | 9 ± 12 - | 3 + 6 - | 6 ± 9 - | 15 + 20 - |
| Phthalic | <i>nor</i> | N/110000 | 4.38 | 2 ^d + 3 ^d - | 36 ^h + 2 ^d - | 2 ^d ± 3 ^d - | 90 ^m + 2 ^h - |
| | <i>iso</i> | N/10000 | " | 3 + 4 - | 2 ^d + 3 ^d - | 3 ± 4 - | 2 ^h + 3 - |
| | <i>tele</i> | N/300000 | " | 6 + 7 - | 4 + 5 - | 5 + 6 - | 9 + 12 - |
| Control | | | | 8 ^d ± | 5 ^d ± | 6 ^d ± | 18 ^h ± |

2.00 respectively. Then I examined the relation between the chemical constitution of cresols, halogen phenols, NO_2 phenols and aromatic acids and the strength of sterilizing action at the same pH solution respectively. Results obtained are as shown in Table 5.

From the above experiments noted in Table 5, I learned the following facts :

1. In the same pH solution the strength of the sterilizing power of cresol is as follows : $m < o < p$.

The strength of the sterilizing action of cresol anion is as follows :

Anions of p cresol have the preventing power for the bacteria but o or m anion has no such power. The variation in the strength of the sterilizing or preventing action of o , m , or p cresol isomer on the bacterial life depends chiefly on the position of CH_3 group combined at benzene ring, but has no relation to pH.

2. The strength of the sterilizing power of halogen phenols and NO_2 phenols in the same pH solution is as follows : $o < m < p$.

The difference between the sterilizing action and the constitution of halogen phenol or NO_2 phenol, chiefly depends on the position of Cl , Br , or NO_2 group combined at benzene ring.

3. When we compare the sterilizing action of benzoic acid and o , m , and p isomers of OH substituted benzoic acid in the same pH solution, we find that there seems to be a great difference in the case of halogen phenols and NO_2 phenols. The order of the sterilizing action is as follows :—benzoic acid $< m$ -OH benzoic acid $< p$ -OH benzoic acid $< o$ -OH benzoic acid. The chief cause of this fact is that the strong sterilizing action of salicylic acid is chiefly due to the low pH, and partly that anion of salicylic acid has sterilizing action. On the other side o -OH benzoic acid and m -OH benzoic acid have high pH compared to salicylic acid and their anions have no sterilizing action on bacteria.

4. If we compare the sterilizing action of 3 isomers of phthalic acids, such as *nor.*, *iso* and *tele.*, we see that the degree of the sterilizing power is *nor.* $>$ *iso* $>$ *tele.* This is due to the chemical constitution of undissociated molecule of each isomer, chiefly position of CO_2H group combined at benzene ring.

§ 5. Summary and discussion concerning the relation between the chemical constitution of phenols and aromatic acids and the physiology of bacteria.

From the results mentioned in the sections (2) to (4), we obtained the following views on the relation between the chemical constitution of phenols and aromatic acids and the sterilizing action on bacteria.

1. The sterilizing action of o , m , and p isomers of phenols, in the same concentration or in the same pH solution, is as follows :

The strongest of all is p isomer, and o isomer is the weakest. e. g., $p > m > o$. But with cresol, the order of the strength of the sterilizing action is as follows : $p > o > m$.

And among OH substituted benzoic acids, the order of the strength of the sterilizing action is as follows :—

o-OH benzoic acid > *p*-OH benzoic acid > *m*-OH benzoic acid > benzoic acid. And in the solution of phthalic acid isomer, normal > iso > tele.

2. The cause of the difference of the sterilizing action of *o*, *m*, and *p* cresol isomer on the bacterial life seems chiefly to depend on the poisoning action for bacterial body by the position of CH₃ group combined at benzene ring. While the difference of the sterilizing action of *o*, *m*, and *p* OH benzoic acids depends chiefly on pH which is changed by the position of OH group combined at benzene ring. Added to this, the difference of the action of undissociated molecules of acid isomers also have some effects on the sterilizing action.

3. The strength of the sterilizing action of phthalic acids in the solution of the same concentration and also in the same pH is as follows:

tele < iso < normal.

The cause of the difference of the strength of each acid isomer depends on the position of CO₂H combined at benzene ring.

4. Judging from the results of the sterilizing action of phenols, OH benzoic acids and phthalic acids, we ascertained the following facts: Anions of phenols, OH benzoic acids and phthalic acids, have generally no sterilizing action, or almost no preventing action on bacteria. Only anions of halogen phenols and NO₂ phenols and *o*-OH benzoic acid have a weak preventing action.

14th Report. On the Relation between the Chemical Constitution of Phenol Isomers and Aromatic Acid Isomers and Adsorption in the Bacterial Body.



Concerning the adequate relation between the strength of the sterilizing action of several phenols and aromatic acids and the adsorption on the bacterial body, we see many reports. But there seems to be no study concerning the difference of chemical constitution having a special effect on the life of bacteria.

I performed this experiment to find how the difference of chemical constitution of phenols, OH benzoic acid isomers and phthalic acid isomers act on the protoplasm of the bacterial body.

(1) Reagents.

Reagents used are as follows:

I. Phenols.

| Phenols | Rational formulae. | Isomer | Constitution formulae | M. P | B P |
|---------|---|----------|---|------|------|
| | Molecular weight | | | | |
| Cresol | C ₆ H ₄ ·CH ₃ ·OH 108 064 | <i>o</i> |  | 30° | 191° |
| | | <i>m</i> |  | 44° | 203° |

clear solution determined its pH by electric method and compare the result with the original pH of each reagent.

Results obtained are as shown in the following table.

Table 3. Chemical constitution of phenols and adsorption on the bacterial body.
(Increasing value of pH).

| Phenols | Isomer | Original pH | Increasing value of pH | |
|-------------------------|----------|-------------|------------------------|---------------|
| | | | Coli communis | Vib. cholerae |
| Cresol | <i>o</i> | 5.36 | 1.03 | 1.12 |
| | <i>m</i> | 5.36 | 0.85 | 0.98 |
| | <i>p</i> | 5.57 | 1.06 | 1.10 |
| Pyrocatechin | <i>o</i> | 5.31 | 1.13 | 1.13 |
| Resorcin | <i>m</i> | 5.57 | 1.27 | 1.35 |
| Hydroquinon | <i>p</i> | 5.64 | 1.25 | 1.28 |
| Pyrogallie acid | <i>o</i> | 4.58 | 1.16 | 1.20 |
| Phloroglucin | <i>m</i> | 5.71 | 1.25 | 1.30 |
| Cl-phenol | <i>o</i> | 5.73 | 0.45 | 0.47 |
| | <i>m</i> | 5.76 | 0.60 | 0.49 |
| | <i>p</i> | 5.76 | 0.80 | 0.89 |
| Br-phenol | <i>o</i> | 5.76 | 0.63 | 0.79 |
| | <i>m</i> | 5.80 | 0.75 | 0.85 |
| | <i>p</i> | 5.80 | 0.93 | 1.15 |
| NO ₂ -phenol | <i>o</i> | 5.76 | 0.65 | 0.87 |
| | <i>m</i> | 5.76 | 0.85 | 1.17 |
| | <i>p</i> | 5.78 | 0.95 | 1.33 |

Table 4. Chemical constitution of aromatic acids and adsorption on the bacterial body, as shown. by increasing pH.

| Aromatic acids | Isomer | Original pH | Increasing pH | |
|---|-----------------|-------------|---------------|---------------|
| | | | Coli communis | Vib. cholerae |
| Benzoic | | 3.58 | 1.58 | 1.85 |
| Salicylic | <i>o</i> | 3.08 | 2.92 | 3.05 |
| <i>m</i> -OH-benzoic | <i>m</i> | 3.68 | 1.85 | 2.79 |
| <i>p</i> -OH-benzoic | <i>p</i> | : | 2.32 | 2.82 |
| Phthalic | normal | 4.34 | 2.32 | 2.38 |
| | iso | 4.38 | 1.97 | 2.04 |
| | tele | 4.13 | 1.79 | 1.89 |
| Cont. 1. HNO ₃ | <i>N</i> /10000 | 4.0 | 2.14 | 2.35 |
| Cont. 2. H ₂ SO ₄ | : | : | : | : |
| Cont. 3. H ₂ O | | 6.33 | 0.70 | 0.73 |

Note: Concentration :—*N*/1000. Phthalic acids = *N*/100000. HNO₃ and H₂SO₄ = *N*/10000.

(3) Discussion and summary of adsorption of aromatic acid isomers and phenol isomers on bacterial bodies.

Relation between the chemical constitution of phenols and aromatic acids and the bacterial life will be shown exactly by studies on the chemical constitution of reagents and the sterilizing, preventing and promoting actions for bacteria. And these three actions have adequate relation to the adsorption on or consumption by the bacterial protoplasm of reagents. I performed this experiment to ascertain how the difference of the chemical constitution of reagents acts on the adsorption on or consumption by the bacterial protoplasm.

By the results noted in the previous section, we can deduce the following conclusions:

(1) The degree of the strength of sterilizing action of phenol isomers and aromatic acid isomers is proportionate to the degree of the quantity adsorbed on the bacterial body. The degree of adsorption has an adequate relation to the position of OH group, Cl and Br or NO₂ group combined at benzene ring in phenol isomers, and CO₂H group combined at benzene ring in OH benzoic acid isomers, respectively.

(2) On the other hand we see that the action of di and tri OH phenol isomers on the bacteria is as follows: *p* and *o* isomers have a sterilizing action and order of the strength is as follows: *o* < *p*. Contrary to this, *m* isomers have a strong promoting action for bacteria. *m* isomers seem to be a nutritive source for bacteria.

(8) The cause of the difference of the sterilizing action of phthalic acid isomers is as follows: We see the difference of the quantity adsorbed on the bacterial body among normal, iso and tele isomers, by the position of CO_2H group combined at benzene ring of phthalic acid.

The difference in the amount of adsorption on bacterial bodies causes the difference of the sterilizing action of phthalic acid isomers.

